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(54) Title: TRANSGENIC CARNATIONS EXHIBIT PROLONGED POST-HARVEST LIFE

(57) Abstract

The present invention relates generally to transgenic plants which exhibit prolonged post-harvest life properties. More particularly, the present invention is directed to transgenic carnation plants modified to reduce expression of one or more enzymes associated with the ethylene biosynthetic pathway. Flowers of such carnation plants do not produce ethylene, or produce ethylene in reduced amounts, and are, therefore, capable of surviving longer post-harvest than flowers of non-genetically modified, naturally-occuring carnation plants.

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# TRANSGENIC CARNATIONS EXHIBIT PROLONGED POST-HARVEST LIFE

The present invention relates generally to transgenic plants which exhibit prolonged post-harvest life properties. More particularly, the present invention is directed to transgenic carnation plants modified to reduce expression of one or more enzymes associated with the ethylene biosynthetic pathway. Flowers of such carnation plants do not produce ethylene, or produce ethylene in reduced amounts, and are, therefore, capable of surviving longer post-harvest than flowers of non-genetically modified, naturally-occurring carnation plants.

Bibliographic details of the publications referred to hereinafter in the specification are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs) referred to herein in relation to nucleotide and amino acid sequences are defined after the Bibliography.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

The flower industry strives to develop new and different varieties of flowering plants, with improved characteristics ranging from disease and pathogen resistance to altered inflorescence and improved post-harvest cut-flower survival. Although classical breeding techniques have been used with some success, improvements in one characteristic are often achieved at the expense of one or more other important characteristics. Recombinant DNA technology provides a means whereby precise improvements are able to be made to one characteristic of a particular cultivar or cultivars, without altering any other commercially-valuable trait. Substantial effort has therefore been directed towards the exploitation of recombinant DNA technology to manipulate the genetic make-up of plants and generate transgenic plants which exhibit desirable characteristics or in which undesirable traits are

suppressed. One of the characteristics most sought after by consumers of cut-flowers is a prolonged post-harvest vase life. The development of longer-living varieties of the major cut-flower species, including for example carnation, would offer a significant opportunity in a cut-flower market with retail sales in excess of US$25 billion.

Flower senescence is associated with the plant's production of ethylene. Ethylene is directly involved in plant growth and development and its production is strictly regulated. The pathway for ethylene biosynthesis in higher plants, as elucidated by Adams and Yang (1979), involves utilization of the endogenous pool of methionine to create S-adenosyl-methionine (SAM) by the enzyme SAM synthetase. SAM is a ubiquitous component of all living cells and is involved in a variety of metabolic processes. The initial step in ethylene biosynthesis occurs when SAM is converted to 1-aminocyclopropane-1-carboxylic acid (ACC) by the enzyme ACC synthase (ACS). This conversion is essential for ethylene production and often constitutes the rate-limiting step in the pathway. The final step is the subsequent conversion of ACC to ethylene by the enzyme ACC oxidase (ACO), also known as Ethylene Forming Enzyme (EFE). Additional information concerning ethylene biosynthesis may be found in a review by Kende (1993).

Regulation of the genes encoding these enzymes determines the temporal and spatial patterns of ethylene biosynthesis. This regulation is complex and varies among different species and different tissues as well as in response to different stimuli. Therefore, the ability to control the level of either of these enzymes, but especially the level of ACC synthase since this enzyme controls the production of ethylene, affords control of ethylene levels and, hence, regulation of plant development characteristics controlled by ethylene. These include seed germination; abscission; stress and wound response; fruit ripening and leaf and flower senescence.

As has been shown in tomato (Rottmann *et al.*; 1991) and *Arabidopsis* (Liang *et al.*; 1992), carnation ACC synthase is encoded by a multigene family (Park *et al.*; 1992), which helps explain the differential regulation of its various isozymes at different developmental stages

in various tissues. Availability of isolated nucleic acid molecules encoding, or complementary to sequences encoding, carnation ACC synthase or ACC oxidase permits the manufacture of recombinant materials, such as genetic constructs, useful for controlling the level of these enzymes in plants. The genetic constructs can be introduced into carnation plants, thereby affording the possibility of regulating the plants' production of ethylene.

Furthermore, availability of isolated nucleic acid molecules encoding particular isozymes of the said enzymes permits the manufacture of genetic constructs which can be introduced into carnation plants and afford the possibility of regulating the production of ethylene in such a way as to produce flowers exhibiting a prolonged post-harvest vase life.

Accordingly, one aspect of the present invention contemplates a method for producing a transgenic plant exhibiting reduced production of climacteric ethylene, compared to its non-transgenic parent or a non-transgenic plant of the same species, said method comprising introducing into a cell or cells of a plant a genetic construct comprising a nucleic acid molecule encoding, or complementary to a sequence encoding ACC synthase or ACC oxidase or a derivative of said nucleic acid molecule, and regenerating a transgenic plant from said cell or cells.

Preferably, the transgenic plant produced by the subject method exhibits one or more of the following properties:

(i) a reduction in production of ACC synthase-specific mRNA or ACC oxidase-specific mRNA;

(ii) a reduction in production of ACC synthase or ACC oxidase enzyme; and/or

(iii) delayed senescence of flowers or flower buds cut from said transgenic plant.

In a related embodiment there is provided a method for producing a transgenic carnation plant, said method comprising introducing into said plant a genetic construct containing an isolated nucleic acid molecule encoding, or complementary to the sequence encoding, ACC synthase or ACC oxidase, or a derivative of said nucleic acid molecule characterized in that

said transgenic plant exhibits one or more of the following properties:

(i) reduction in the production of ACC synthase-specific mRNA or ACC oxidase-specific mRNA;

(ii) reduction in the production of ACC synthase or ACC oxidase enzyme;

(iii) reduction in the production of climacteric ethylene; and/or

(iv) delayed senescence.

Even more particularly, the present invention contemplates a method for producing a transgenic carnation plant exhibiting prolonged post-harvest life properties, said method comprising introducing into said carnation plant a genetic construct comprising a non-full-length fragment of a nucleic acid molecule encoding ACC synthase or ACC oxidase.

By "climacteric" ethylene is meant the developmentally-regulated production of ethylene which induces a series of chemical events leading to ripening or senescence of an organ. The term was originally used to describe the metabolic state of ripening fruit, but also applies to the senescence of carnation flowers. A peak of production of climacteric ethylene by a control plant can be readily seen in Figure 9.

Preferably, the non-full-length fragment is approximately 800-1200 base-pair in length. Preferably, the non-full-length fragment is an internal fragment of the nucleic acid molecule encoding ACC synthase or ACC oxidase.

Preferably, the non-full-length fragment is inserted in the sense orientation such that reduction of ACC synthase or ACC oxidase expression is by co-suppression.

The genetic constructs of the present invention comprise an isolated nucleic acid molecule encoding, or complementary to the sequence encoding, ACC synthase or ACC oxidase, or a derivative of said nucleic acid molecule and where necessary comprise additional genetic sequences such as promoter and terminator sequences which regulates expression of the molecule in the transgenic plants. When the genetic construct is DNA it may be cDNA or

genomic DNA. The ACC synthase or ACC oxidase genetic sequences are preferably from carnation plants. However, the present invention extends to similar genetic sequences from other plants such as related flowering plants and which have a genetic sequence capable of acting via antisense or co-suppression methods.

By "nucleic acid molecule" as used herein is meant any contiguous series of nucleotide bases specifying a sequence of amino acids in ACC synthase or ACC oxidase. The nucleic acid may encode the full-length enzyme or a derivative thereof. Furthermore, the nucleic acid molecule may not encode a full-length ACC synthase or ACC oxidase but is of sufficient length to down regulate an endogenous ACC synthase or ACC oxidase gene by co-suppression or antisense. By "derivative" is meant any single or multiple amino acid substitutions, deletions, and/or additions relative to the naturally-occurring enzyme. In this regard, the nucleic acid includes the naturally-occurring nucleotide sequence encoding ACC synthase or ACC oxidase or may contain single or multiple nucleotide substitutions, deletions and/or additions to said naturally-occurring sequence. The terms "analogues" and "derivatives" also extend to any chemical equivalent of the ACC synthase or ACC oxidase, the only requirement of the said nucleic acid molecule being that when used to produce a transgenic plant in accordance with the present invention said transgenic plant exhibits one or more of the following properties:

(i) reduction in the production of ACC synthase-specific mRNA or ACC oxidase-specific mRNA;

(ii) reduction in the production of ACC synthase or ACC oxidase enzyme;

(iii) reduction in the production of climacteric ethylene; and/or

(iv) delayed senescence.

A derivative of the subject nucleic acid molecule is also considered to encompass a genetic molecule capable of hybridising to the nucleotide sequence set forth in SEQ ID NO:3 under low stringency conditions at 30°C. Reference to low stringency conditions includes hybridising DNA with 50% formamide at 30°C. Alternative conditions such as medium and high stringency conditions may also be employed depending on the derivative.

More particularly, the transgenic carnation plant carries flowers or flower buds which, when cut from the carnation plant, exhibit prolonged post-harvest life properties as well as one or more of the following properties:

(i) reduced levels of ACC synthase-specific mRNA or ACC oxidase below non-transgenic endogenous levels;

(ii) reduced levels of ACC synthase or ACC oxidase enzyme below non-transgenic endogenous levels; and/or

(iii) reduced levels of climacteric ethylene production below non-transgenic endogenous levels;

In a preferred embodiment of the present invention, there is provided a method for producing transgenic carnation plants, said method comprising introducing into said plants a genetic construct containing an isolated nucleic acid molecule encoding, or complementary to the sequence encoding, a non-full-length portion of ACC synthase or ACC oxidase, characterized in that the flowers of the said transgenic plants exhibit one or more of the following properties:

(i) reduction in the production of ACC synthase-specific mRNA or ACC oxidase-specific mRNA;

(ii) reduction in the production of ACC synthase or ACC oxidase enzyme;

(iii) reduction in the production of climacteric ethylene; and/or

(iv) delayed senescence.

The present invention further extends to such transgenic plants having one or more of the above-mentioned properties and to cut flowers or cut parts from said plants including flower buds from said plants.

More particularly, the flowers of the said transgenic plants exhibit one or more of the following properties:

(i) reduced levels of ACC synthase-specific mRNA or ACC oxidase-specific mRNA below non-transgenic endogenous levels;

(ii) reduced levels of ACC synthase or ACC oxidase enzyme below non-transgenic endogenous levels;

(iii) reduced levels of climacteric ethylene production below non-transgenic endogenous levels; and/or

(iv) delayed senescence.

Reference herein to the level of ACC synthase enzyme relates to a reduction of 30% or more, or more preferably of 30-50%, or even more preferably 50-75% or still more preferably 75% or greater below the normal endogenous or existing levels of enzyme. Such reduction may be referred to as "modulation" of ACC synthase or ACC oxidase enzyme activity. It is possible that modulation is at the level of transcription, post-transcriptional stability or translation of the ACC synthase or ACC oxidase genetic sequences.

The nucleic acid molecules used herein may exist alone or in combination with a vector molecule and preferably an expression-vector. Such vector molecules replicate and/or express in eukaryotic and/or prokaryotic cells. Preferably, the vector molecules or parts thereof are capable of integration into the plant genome. The nucleic acid molecule may additionally contain a sequence useful in facilitating said integration and/or a promoter sequence capable of directing expression of the nucleic acid molecule in a plant cell. The nucleic acid molecule and promoter may be introduced into the cell by any number of means such as by electroporation, micro-projectile bombardment or *Agrobacterium*-mediated transfer.

Accordingly, another aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides encoding, or complementary to a sequence encoding a carnation ACC synthase or ACC oxidase or a mutant, derivative, part, fragment, homologue or analogue of said ACC synthase or ACC oxidase. In one embodiment, such mutants may also be functional, meaning that they exhibit at least some ACC synthase or ACC oxidase activity. In all cases, the nucleic acid molecules are capable of suppressing ACO or ACS gene expression, mediated by the nucleic acid molecule being in one or the

other orientation relative to its or another promoter; i.e. by sense suppression or antisense suppression. The expressions "ACC synthase" and "ACC oxidase" include reference to polypeptides and proteins having ACC synthase or ACC oxidase activity as well as any mutants, derivatives, parts, fragments, homologues or analogues of such polypeptides or proteins and which have ACC synthase or ACC oxidase activity. A molecule having ACC synthase or ACC oxidase activity may also be a fusion polypeptide or protein between a polypeptide or protein having ACC synthase or ACC oxidase activity and an extraneous peptide, polypeptide or protein.

As used herein, the term "isolated nucleic acid molecule" is meant to include a genetic sequence in a non-naturally-occurring condition. Generally, this means isolated away from its natural state or formed by procedures not necessarily encountered in its natural environment. More specifically, it includes nucleic acid molecules formed or maintained *in vitro*, including genomic DNA fragments, recombinant or synthetic molecules and nucleic acids in combination with heterologous nucleic acids such as heterologous nucleic acids fused or operably-linked to the genetic sequences of the present invention. The term "isolated nucleic acid molecule" also extends to the genomic DNA or cDNA, or part thereof constituting ACC synthase or ACC oxidase or a mutant, derivative, part, fragment, homologue or analogue of ACC synthase or ACC oxidase, whether in sense or in reverse orientation relative to its or another promoter. It further extends to naturally-occurring sequences following at least a partial purification relative to other nucleic acid sequences. The term "isolated nucleic acid molecule" as used herein is understood to have the same meaning as a "nucleic acid isolate". In a particular embodiment, mutants and other like variants of ACC synthase or ACC oxidase retain at least some ACC synthase or ACC oxidase activity and are therefore considered functional.

The expression "genetic sequences" is used herein in its most general sense and encompasses any contiguous series of nucleotide bases specifying directly, or via a complementary series of bases, a sequence of amino acids comprising an ACC synthase or ACC oxidase molecule including a polypeptide or protein having ACC synthase or ACC oxidase activity. Such a

sequence of amino acids may constitute a full-length ACC synthase such as is set forth in, for example, SEQ ID NO:3 or a truncated form thereof or a mutant, derivative, part, fragment, homologue or analogue thereof. Alternatively, the amino acid sequence may comprise part of, for example, these sequences or all or part of the sequences set forth in SEQ ID NO:3, as can be seen in SEQ ID NO:4. The amino acid sequence may alternatively constitute ACC oxidase as set forth in SEQ ID NO:7. The present invention encompasses nucleic acid molecules encoding the above-mentioned amino acid sequences as well as nucleic acid molecules encoding amino acid sequences having at least about 60%, more preferably about 70%, even more preferably about 80%, and still more preferably about 90%, or above, similarity to the amino acid sequences set forth in either SEQ ID NO:3 or SEQ ID NO:7.

In accordance with the present invention, a nucleic acid molecule encoding, or complementary to the sequence encoding, ACC synthase or ACC oxidase may be introduced into and expressed in a transgenic carnation, thereby providing a means whereby the production of climacteric ethylene by flowers of the said plant may be reduced to below naturally-occurring levels. This allows the onset of flower senescence to be prevented or delayed and flowers to exhibit a prolonged vase life following harvest. Background information on antisense and sense suppression technologies can be found in US Patent Number 5,107,065 and in US Patent Numbers 5,034,323; 5,231,020 and 5,283,184, respectively.

Accordingly, the present invention provides a method for producing a transgenic flowering plant wherein the flowers exhibit reduced levels of ethylene production below non-transgenic levels, said method comprising introducing into a cell of a carnation plant, a genetic construct comprising a nucleic acid molecule encoding, or complementary to the sequence encoding, ACC synthase or ACC oxidase under conditions permitting the integration of said nucleic acid molecule into the plant's genome, regenerating a transgenic plant from the cell and growing said transgenic plant for a time and under conditions sufficient to permit the transcription of the nucleic acid molecule into the ACC synthase-specific mRNA or ACC oxidase-specific mRNA and, if necessary, the further translation of the ACC synthase mRNA or ACC oxidase-specific mRNA into the enzyme ACC synthase or ACC oxidase. Preferably, the introduced genetic

construct comprises a non-full-length segment of a nucleic acid molecule encoding ACC synthase or ACC oxidase. This aspect of the present invention extends to flowers cut or otherwise severed from said transgenic plants, including parts of flowers and parts of transgenic plants carrying flowers or flower buds.

The present invention further extends to functionally-equivalent methods for achieving the production of a transgenic carnation plant and flowers therefrom exhibiting the said characteristics.

The present invention is exemplified by generation of transgenic carnation plants of the varieties Red Corso; Ember Rose; Crowley Sim; White Sim; Scania, containing introduced ACC synthase and/or ACC oxidase genetic sequences. The use of these cultivars in no way limits the applicability of the invention described herein, and the results obtained from these transgenic cultivars are generally applicable to other carnation cultivars.

In a preferred embodiment, the transgenic carnation plant produces flowers which exhibit delayed senescence properties coincident with reduced levels of climacteric ethylene production. Consequently, the present invention extends to a transgenic carnation plant containing all or part of a nucleic acid molecule representing ACC synthase or ACC oxidase and/or any homologues or related forms thereof and in particular those transgenic plants which produce flowers exhibiting reduced ACC synthase- or ACC oxidase-specific mRNA and/or reduced ACC synthase or ACC oxidase levels and/or reduced ethylene production and/or delayed senescence properties. The transgenic plants, therefore, contain a stably-introduced nucleic acid molecule comprising a nucleotide sequence encoding the ACC synthase or ACC oxidase enzyme. The invention extends to flowers cut from such transgenic plants and to seeds derived from same.

Another aspect of the present invention is directed to a prokaryotic or eukaryotic organism carrying a genetic sequence encoding an ACC synthase or ACC oxidase extrachromasomally in plasmid form. In one embodiment, the plasmid is pWTT2160 in *Agrobacterium tumefaciens*.

In a further embodiment, the plasmid is pCGP407 in *Escherichia coli*. The microorganisms *Escherichia coli* strain XL1-Blue and *Agrobacterium tumefaciens* strain EHA101 containing the plasmids pCGP407 and pWTT2160, respectively, were deposited with the Australian Government Analytical Laboratories, 1 Suakin Street, Pymble, New South Wales, 2037, Australia on May 1, 1995 under Accession Numbers N95/26121 and N95/26122, respectively.

The present invention is further described by reference to the following non-limiting Figures and Examples.

In the Figures:

**Figure 1** is an alignment of nucleotide sequences for ACC synthase-encoding cDNAs from a variety of species. Carnation sequences from cultivars White Sim and Scania are compared with sequences from petunia (EMBL accession number Z18952); tomato (van der Straeten *et al.*, 1990); orchid (Genbank accession number L07882); *Arabidopsis thaliana* (Liang *et al.*, 1992) and zucchini (Sato *et al.*, 1991). Alignments were performed for the coding regions of the sequences using the Clustal V programme of Higgins *et al.*, 1991. Translation initiation and termination codons are underlined. Asterisks indicate conserved nucleotides.

**Figure 2** is a diagrammatic representation of the binary expression vector pWTT2160, construction of which is described in Example 4. Tc resistance = the tetracycline resistance gene; LB = left border; RB = right border; *Sur*B = the coding region and terminator sequences for the acetolactate synthase gene; 35S = the promoter region from the cauliflower mosaic virus 35S gene; car ACS = the nucleic acid molecule encoding carnation ACC synthase; nos 3' = the terminator region from the *Agrobacterium tumefaciens* nopaline synthase gene. Selected restriction enzyme sites are indicated.

Figure 3 is an alignment of nucleotide sequences for ACC oxidase-encoding cDNAs from a variety of plant species. Carnation sequences from cultivars Scania and White Sim are compared with sequences from *Arabidopsis thaliana*, tomato (Holdsworth *et al.*, 1987; EMBL

accession number X 04792); orchid (Nadeau *et al.*, 1993; Genbank accession number L 07912); apple (Dong *et al.*, 1992); petunia (Wang and Woodson, 1992); sunflower (Liu and Reid, unpublished; Genbank accession number L 29405) and geranium (Wang *et al.*, 1994). Alignments were performed for the coding regions of the sequences using the Clustal V programme of Higgins *et al.*, 1991. Translation initiation and termination codons are underlined. Asterisks indicate conserved nucleotides. Asterisks indicate conserved nucleotides.

**Figure 4** is a diagrammatic representation of the binary expression vector pCGP407, construction of which is described in Example 8. Gm = the gentamycin resistance gene; RB = right border; LB = left border; car ACO = the nucleic acid molecule encoding carnation ACC oxidase: MAC = the mannopine synthase promoter enhanced with cauliflower mosaic virus 35S gene sequences; mas 3' = the terminator region from the *Agrobacterium tumefaciens* mannopine synthase gene; 35S = the promoter region form the cauliflower mosaic virus 35S gene; NPT II = neomycin phosphotransferase II; tml 3' = the tml terminator region, DNA sequences 11207-10069, from pT;A6 (Barker *et al.*, 1983). Selected restriction enzyme sites are indicated.

**Figure 5** is an autoradiographic representation of a Southern hybridization of DNA isolated from leaf tissue from a number of different carnation cultivars, which had been transformed with a genetic construct (pWTT2160) containing the acetolactate synthase gene (ALS), as selectable marker, and an internal fragment of the nucleic acid molecule encoding ACC synthase. Carnation genomic DNA was digested with *Eco*RI and the Southern blot was probed with a $^{32}$P-labelled-760 base pair fragment derived from the ALS coding region. Filters were washed in 0.2 x SSC/1% w/v SDS at 65°C. Numbers 1-4 represent cultivars White Sim; Crowley Sim; Ember Rose and Scania, respectively. The negative control (N) is non-transformed White Sim. Multiple bands in lanes 1-4 indicate where copies of DNA derived from pWTT2160 have been integrated into the genome of plants. No bands were detected in the non-transformed negative control.

Figure 6 is an autoradiographic representation of a Southern hybridization of DNA isolated from leaf tissue from the carnation cultivars White Sim and Scania, which had been transformed with a genetic construct (pCGP407) containing the neomycin phosphotransferase (NPT II) gene as selectable marker, and a nucleic acid molecule defining ACC oxidase, in reverse orientation relative to the promoter. Carnation genomic DNA was digested with the restriction enzyme *Hind* III. The Southern blot was probed with a $^{32}$P-labelled *Eco*RI DNA fragment from the coding sequence of the NPT II gene. Filters were washed in 0.1 x SSC, 0.1% w/v SDS at 65°C. The bands indicate single or multiple copies of the DNA derived from pCGP407 have been integrated into the genome of the plants. In lane 2, the Scania plant #705 shows 6 copies of the NPT II gene and White Sim plant #2373B, in lane 5, has a single copy of NPT II. No bands were detected in the non-transformed negative control. The size of the fragments detected is indicated in kilobases on the left-hand side of the figure.

Figure 7 is an autoradiographic representation of a Northern blot of RNA isolated from lateral shoot tissue from carnations transformed with pWTT2160. The control is non-transformed White Sim. Eight independent transgenic lines are shown. Filters were probed with a $^{32}$P-labelled *Hind*III DNA fragment from the acetolactate synthase gene coding region, and washed for 30 min in 2 x SSC, 1% w/v SDS at 65°C, followed by 2 x 30 min in 0.2 x SSC, 1% w/v SDS at 65°C.

Figure 8 is an autoradiographic representation of a Northern blot of ACC oxidase mRNA and ACC oxidase antisense RNA isolated from petals. Total RNA (10$\mu$g/lane) was analysed from day 0 petals of control, non-transgenic White Sim (lane 1), transgenic Scania (lane 3) and transgenic White Sim (lane 5) flowers; and day 5 petals of control, non-transgenic White Sim (lane 2), transgenic Scania (lane 4) and transgenic White Sim (lane 6) flowers. Also analysed was total RNA isolated from transgenic Scania (lane 7), transgenic White Sim (lane 8) day 5 flowers which had been exposed to ethylene (150ppm) for the preceding 18 h. Filters were hybridised with either a strand-specific antisense RNA probe, to detect ACC oxidase mRNA, or a strand-specific sense ACC oxidase RNA probe to detect antisense ACC

oxidase RNA, and washed in 2 x SSC/1% w/v SDS at 65°C for 1 hour followed by 0.2 x SSC/1% w/v SDS at 65°C for 1 hour and, in the case of antisense ACO, finally in 0.1 x SSC/0.1% w/v SDS at 65°C for 1 hour. Ribonuclease treatment was incorporated.

**Figure 9** shows a graph of ethylene production in carnation flowers. Flowers of carnation cvs. Scania and White Sim were placed in a gas-tight chamber for three hours each day after harvest. The ethylene content of a gas sample taken from the chamber was measured using gas chromatography, as described in Example 19. Ethylene measurements are expressed as nanolitres of ethylene produced per gram of flower tissue (not including stem) per hour. Values for the control, non-transgenic flowers are the average of ethylene measurements from nine individual flowers. The transgenic Scania and White Sim values are averaged from 3 flowers each.

**Figure 10(A)-10(F)** is a black and white reproduction of colour photographic plates representing a:

(A) non-transgenic control Scania flower, 0 days post-harvest;

(B) non-transgenic control Scania flower, 4 days post-harvest;

(C) non-transgenic control Scania flower, 7 days post-harvest;

(D) transgenic ACC synthase sense-suppressed Scania flower, 0 days post-harvest;

(E) transgenic ACC synthase sense-suppressed Scania flower, 4 days post-harvest; and

(F) transgenic ACC synthase sense-suppressed Scania flower, 11 days post-harvest.

The transgenic flower remains fresh at 11 days post-harvest, while the non-transgenic control has inrolled by day 4 and is completely senesced by 7 days post-harvest. Original colour plates are available for inspection from the Applicant.

**Figure 11(A)-11(F)** is a black and white reproduction of colour photographic plates representing a:

(A) non-transgenic control Red Corso flower, 0 days post-harvest;

(B) non-transgenic control Red Corso flower, 7 days post-harvest;

(C) non-transgenic control Red Corso flower, 9 days post-harvest;

(D) transgenic ACC synthase sense-suppressed Red Corso flower, 0 days post-harvest;

(E) transgenic ACC synthase sense-suppressed Red Corso flower, 7 days post-harvest; and

(F) transgenic ACC synthase sense-suppressed Red Corso flower, 9 days post-harvest.

The transgenic flower remains fresh at 9 days post-harvest, while the non-transgenic control has inrolled and completely senesced by 7 days post-harvest. Original colour plates are available for inspection from the Applicant.

**Figure 12(A)-12(F)** is a black and white reproduction of colour photographic plates representing a:

(A) non-transgenic control Ember Rose flower, 0 days post-harvest;

(B) non-transgenic control Ember Rose flower, 4 days post-harvest;

(C) non-transgenic control Ember Rose flower, 7 days post-harvest;

(D) transgenic ACC synthase sense-suppressed Ember Rose flower, 0 days post-harvest;

(E) transgenic ACC synthase sense-suppressed Ember Rose flower, 4 days post-harvest; and

(F) transgenic ACC synthase sense-suppressed Ember Rose flower, 7 days post-harvest.

Original colour plates are available for inspection from the Applicant.

**Figure 13(A)-13(D)** is a black and white reproduction of colour photographic plates representing a:

(A) non-transgenic control Crowley Sim flower, 0 days post-harvest;

(B) non-transgenic control Crowley Sim flower, 4 days post-harvest;

(C) transgenic ACC synthase sense-suppressed Crowley Sim flower, 0 days post-harvest; and

(D) transgenic ACC synthase sense-suppressed Crowley Sim flower, 4 days post-harvest.

Original colour plates are available for inspection from the Applicant.

**Figure 14(A)-14(C)** is a black and white reproduction of colour photographic plates representing:

(A) one non-transgenic control White Sim flower (on the left of the photograph), and three ACC synthase sense-suppressed transgenic flowers at 0 days post-harvest;

(B) one non-transgenic control White Sim flower (on the left of the photograph), and three ACC synthase sense-suppressed transgenic flowers at 11 days post-harvest; and

(C) one non-transgenic control White Sim flower (on the left of the photograph), and three ACC synthase sense-suppressed transgenic flowers at 20 days post-harvest.

All flowers were kept in distilled water and under controlled light and temperature conditions following harvest. The non-transgenic control flower has inrolled and is senescing by 11 days post-harvest and is completely senesced by 20 days post-harvest, while the control flowers remain fresh at 20 days post-harvest. Original colour plates are available for inspection from the Applicant.

**Figure 15** is a black and white reproduction of a colour photographic plate representing one non-transgenic control Scania flower (on the left of the photograph), and one antisense ACC oxidase transgenic Scania flower, taken at 6 days post-harvest. Vase life measurements were carried out in distilled water and under controlled light and temperature conditions. An original colour plate is available for inspection from the Applicant.

**Figure 16** is a black and white reproduction of a colour photographic plate representing one non-transgenic control White Sim flower (on the right of the photograph), and one antisense ACC oxidase transgenic White Sim flower, taken at 8 days post-harvest. The flowers were kept in distilled water and under controlled light and temperature conditions following harvest. An original colour plate is available for inspection from the Applicant.

## EXAMPLE 1

### Biological Reagents

All restriction enzymes and other reagents were obtained from commercial sources and used generally according to the manufacturer's recommendations.

The cloning vector pBluescript II (KS+) was obtained from Stratagene.

## EXAMPLE 2

### Bacterial Strains

The bacterial strains used were:

*Escherichia coli* :

XL1-Blue supE44, hsdR17 ($r_k$-, $m_k$+), recA1, endA1, gyrA96 ($Nal^r$), thi-1, relA1, lac-, [F'proAB, $lacI^q$, lacZΔM15, Tn10($tet^r$)] (Bullock *et al.*,1987).

DH5α supE44 Δ(lacZYA-ArgF)U169 ø80dlacZΔM15 hsdR17($r_k$-, $m_k$+), recA1, endA1, gyrA96 ($Nal^r$), thi-1, relA1, deoR (Hanahan, 1983 and BRL, 1986).

JM 83 F araΔ(lac-proAB) rpsL ($Str^r$)[ø80dΔ(lacZ)M15] (Vieira and Messing, 1982)

JM 109 F'traD36 lac $I^q$ Δ(lacZ)M15, proA+B+/e14-(McrA-) Δ(lac-proAB) thi gyrA96 ($Nal^r$) endA1 hsdR17 ($r_k$-, $m_k$+) relA1 supE44 recA1 (Yanisch-Perron *et al.*, 1985)

*Agrobacterium tumefaciens* :

AGL0 Lazo *et al.* (1991)

EHA101 Hood *et al.* (1984)

## EXAMPLE 3

### Growth Conditi ns

Unless otherwise stated, plants were grown in specialised growth rooms with a 14 h day length at a light intensity of 10,000 lux minimum and a temperature of 22 to 26°C.

## EXAMPLE 4

### Isolation of a carnation ACC synthase (ACS) clone from cv. White Sim

*a. Polymerase Chain Reaction Primers*

A carnation ACC synthase (ACS) cDNA clone from cv. White Sim was prepared using a reverse-transcriptase Polymerase Chain Reaction (PCR) method. PCR primers were synthesized based on highly-conserved regions occurring within the approximately 1,500 base pair (bp) coding sequence. An approximately 1,100 bp fragment was obtained after amplification. The primer sequences employed were :

5' ATGGGT(C/T)TNGCNGAAAATCAGC 3' SEQ ID NO:1
5' A(G/A)CANACNCG(A/G)AACCANCCNGG 3' SEQ ID NO:2

*b. Isolation of an ACS clone from carnation flowers*

RNA was isolated from carnation cv. White Sim petals harvested at the fully open stage and then exposed to 1 part per million ethylene overnight to induce climacteric ethylene synthesis. A standard phenol lysis method was used for the RNA isolation (Jones *et al*, 1985). PolyA$^+$ RNA was prepared from the total RNA preparation using standard oligo(dT) cellulose chromatography (Aviv and Leder, 1972). The reverse-transcriptase reaction and subsequent PCR amplification were performed according to Ausubel *et al.* , 1992. A fragment of the predicted size of approximately 1,100 bp was obtained after reverse-transcriptase-PCR of PolyA$^+$ RNA from ethylene-treated carnation flowers.

## EXAMPLE 5

### Sequence analysis of carnation cv. White Sim ACS cDNA clone

The approximately 1,100 bp carnation ACS cDNA fragment was cloned into the vector pBluescript II (KS+) and the terminal nucleotides were sequenced using SEQ ID NO:1 and SEQ ID NO:2 oligonucleotides as sequencing primers. DNA sequencing was performed essentially by the method of Sanger *et al.* (1977) using the Sequenase enzyme (USB, version 2.1), and showed this approximately 1,100 bp fragment to be part of the climacteric ACS gene of carnation, based on nucleotide sequence similarity to the sequence from Park *et al.*

(1992). The full-length carnation ACS nucleotide sequence is presented as SEQ ID NO:3 and the approximately 1,100 bp internal fragment is presented as SEQ ID NO:4.

## EXAMPLE 6

### Isolation of a carnation ACC synthase (ACS) clone from cv. Scania

An alternative approach was used to isolate another ACS cDNA clone, this time from the cultivar Scania.

*a. Polymerase Chain Reaction Primers*

A petunia ACC synthase cDNA fragment from cv. Old Glory Blue was prepared using PCR. Primers were synthesized based on known coding sequence from the tomato ACS cDNA, pcVV4A, of van der Straeten *et al.* (1990). The primer sequences employed were:

5' CGGGATCCGCTACTAATGAAGAGCATGGC 3' SEQ ID NO:5
5' GCGGTACCAGGTGACGAAAGTGGTGACA 3' SEQ ID NO:6

*b. Isolation of an ACS clone from petunia flowers*

RNA was isolated from petunia cv. Old Glory Blue senescing flower petals which were producing greater than 5 nL ethylene/gram fresh weight/hour. A standard CsCl cushion method (Sambrook *et al.*, 1989) was used for the RNA isolation. The reverse-transcriptase reaction and subsequent PCR amplification were performed according to Ausubel *et al.*, 1992. A 1,380 bp fragment was obtained after 35 amplification cycles. Determination of the nucleotide sequence of the PCR product confirmed that it encoded a polypeptide similar to the deduced translation product of the corresponding region from tomato pcVV4A cDNA.

*c. Construction of a carnation cv. Scania cDNA library*

A cDNA library was constructed using mRNA from senescing carnation petals of the cv. Scania and the Lambda ZAP cDNA cloning vector (Stratagene). The cDNA was generated by oligo(dT) priming of PolyA$^+$-enriched RNA using Maloney's Murine Leukaemia Virus Reverse Transcriptase (MMLV) (BRL). The second strand of cDNA was produced with

DNA Polymerase I (Klenow fragment), blunted, and linkers were added to create *Eco*RI-compatible ends. This DNA was then size-selected on a S200 column (Pharmacia) and ligated into Lambda ZAP bacteriophage arms to create a library with 60,000 recombinant phage. This library was amplified to provide a working stock (Sambrook *et al.* 1989).

*d. Heterologous screening of carnation cDNA library*

A 1,380 bp petunia ACC synthase- encoding PCR fragment was $^{32}$P-labelled and used to screen the 60,000 plaques of the senescing carnation cv. Scania petal cDNA library (Example 6c., above), under conditions of low stringency: the filters were hybridized in 50% formamide at 30°C, and washed for 30 min in 5 x SSC, 1% w/v SDS at room temperature, followed by 2 x 30 min in 5 x SSC, 1% w/v SDS at 42°C.

From the heterologous screening, 10 cDNA clones were isolated. Analysis of five of these clones showed that they all represented the same gene. The longest of the clones contained an insert of approximately 1,820 bp.

## EXAMPLE 7

### Sequence analysis of carnation cv. Scania ACS cDNA clone

The longest clone, approximately 1,820 bp, was sequenced on both strands. It was found to be 99.6% similar to the nucleotide sequence of the cDNA encoding ACC synthase from carnation cv. White Sim, isolated by Park *et al.* (1992) (see Example 5, above). The Scania sequence is 133 bp shorter and contains several nucleotide differences, leading to three amino acid changes: serine to glycine at position 131; arginine to glycine at position 381; isoleucine to serine at position 500. It also contains an additional threonine at position 130.

Homology searches against Genbank, SWISS-PROT and EMBL databases were performed using the FASTA and LFASTA programmes (Pearson and Lipman, 1988). Alignment and comparison of the carnation cv.s White Sim and Scania ACC synthase sequences with five other sequences as follows: petunia; tomato; orchid; arabidopsis; zucchini, can be seen in Figure 1. Alignments were performed using the Clustal V programme (Higgins and Sharp,

1989; Higgins *et al*, 1991). Percentage similarities ranged from 99.6%, between the carnation cultivars, to 65.1% between carnation and zucchini.

## EXAMPLE 8

### Construction of pWTT2160

The 1,100 bp carnation cv. White Sim ACS cDNA fragment (see Example 5) was inserted between a cauliflower mosaic virus 35S promoter/chlorophyll ab binding protein (Cab) 5' region and the nopaline synthase 3' region (Harpster *et al.*, 1988). The resulting fragment comprising a chimaeric, partial carnation ACS genetic sequence was inserted into T-DNA vectors containing a suitable selectable marker gene, such as one which comprises the 35S promoter together with the *Sur*B gene (tobacco acetolactate synthase) allowing selection of chlorsulfuron-resistant transformants. One such resulting vector was given the designation pWTT2160, and is shown in Figure 2.

## EXAMPLE 9

### Transformation of *E. coli* and *A. tumefaciens* with pWTT2160

*Escherichia coli* strains JM 83 (Vieira and Messing, 1982) and JM 109 (Yanisch-Perron *et al.*, 1985), used for routine manipulations, were transformed according to standard procedures (Sambrook *et al.*, 1989) .

To transfer the binary vector pWTT2160 (see Figure 2) from *E. coli* to *Agrobacterium tumefaciens* strain EHA101, the technique of triparental mating (Ditta *et al.*, 1980) was used. *E. coli* strain NE 47, containing the mobilizing plasmid pRK 2013 (Gutterson *et al.*, 1986), was the helper strain. The EHA101 strain was rifampicin-resistant (Hood *et al.*, 1984), enabling transconjugants to be selected on LB-agar plates (Ausubel *et al.*, 1992) containing 10 μg/mL gentamycin and 100μg/mL rifampicin at 28°C.

## EXAMPLE 10

### Transformation of *Dianthus caryophyllus* with partial ACC synthase sequence

*a. Plant Material*

*Dianthus caryophyllus* (cvs. Crowley Sim, Scania, Dark Pierrot, Ember Rose, Laguna, Mango, Monte Lisa, Red Corso, Tangerine, Valencia and Ashley) cuttings were obtained from Van Wyk and Son Flower Supply, Victoria, Australia. The outer leaves were removed and the cuttings were sterilized briefly in 70% v/v ethanol followed by 1.25% w/v sodium hypochlorite (with Tween 20) for 6 min and rinsed three times with sterile water. All the visible leaves and axillary buds were removed under the dissecting microscope before co-cultivation.

For cv. White Sim, stems grown in the greenhouse were harvested, surface-sterilized for 2 min in 75% v/v ethanol followed by 20% v/v commercial bleach + 0.1% v/v Tween-20 for 20 - 30 min, and rinsed three times in sterile water. Shoot tip meristems were isolated, nodes of approximately 1 cm in length were cut from the stem, and both were cultured, at a density of 10-12/standard Petri dish, on a shoot multiplication medium consisting of Murashige and Skoog's (1962) medium (MS) supplemented with B5 vitamins (Gamborg *et al.*, 1968); 590 mg/L 2-[N-morpholino] ethane sulphonate (MES); 1 mg/L benzylaminopurine (BAP); 0.02 mg/L α-naphthalene acetic acid (NAA); 30g/L sucrose; 0.25 % w/v Gelrite Gellan Gum (Schweizerhall), pH 5.8. All phytohormones were added after autoclaving. Cultures were incubated in a growth chamber with a 16-hour photoperiod (~30 $\mu E/m^2/s$) at 24 ± 1° C. The light source was always above the cultures, as heat from light below caused condensation and resulted in poor regeneration and multiplication. Each meristem produced a few vitrified shoots within two weeks. These were excised and sub-cultured monthly on fresh shoot multiplication medium. After 3-4 sub-cultures, shoot cultures which multiplied at a high rate were established; i.e.: each shoot with 3-4 leaves produced a cluster of shoots with a total of 20-25 leaves within a month. These were used routinely as a source of leaf explants for transformation.

*b. Co-cultivation of Agrobacterium and Dianthus Tissue*

*Agrobacterium tumefaciens* strain AGL0 (Lazo *et al.*, 1991), containing the binary vector pWTT2160, was maintained at 4°C on LB agar plates with 50 mg/L tetracycline. A single colony was grown overnight in liquid LB broth containing 50 mg/L tetracycline. The following day it was diluted to 5 x $10^8$ cells/mL with liquid MS medium, before inoculation. Acetosyringone was added to the *Agrobacterium* suspension to a final concentration of 20$\mu$M. *Dianthus* stem tissue was co-cultivated with *Agrobacterium* for 5 days on MS medium supplemented with 3% w/v sucrose, 0.5 mg/L BAP, 0.5 mg/L 2,4-dichlorophenoxy-acetic acid (2,4-D), 100 $\mu$M acetosyringone and 0.25% w/v Gelrite (pH 5.7).

For co-cultivation with the *Dianthus* cultivar White Sim, *Agrobacterium tumefaciens* strain EHA101 (Hood *et al.*, 1984) containing the binary vector pWTT2160 was taken from frozen samples in glycerol, cultured for 2 days at 28°C in the dark on solid L-broth (Ausubel *et al.*, 1992) containing the appropriate antibiotics for selection, and suspended overnight in liquid MinA (Ausubel *et al.* , 1992) for inoculation. Bacterial concentration for inoculation of plant tissue was 0.5 - 1.0 x $10^9$ cells/mL. Acetosyringone was added to the *Agrobacterium* suspension to a final concentration of 20$\mu$M.

Leaves of the cultivar White Sim were isolated by pulling from shoot cultures. For selection with chlorsulfuron it was advantageous to remove only the axillary meristems larger than 1 mm. Leaves were mixed with bacteria for a few minutes, then taken off the mixture and placed on a filter paper on a co-cultivation medium for 5 days. The co-cultivation medium was the same as the shoot multiplication medium but contained 0.5 mg/L BAP and 0.5 mg/L 2,4-D instead of 1 mg/L BAP; 0.02 mg/L NAA, as well as 100$\mu$M acetosyringone. Plates were sealed with parafilm.

*c. Recovery of Transgenic Dianthus Plants*

For selection of transformed stem tissue, the top 6-8 mm of each co-cultivated stem was cut into 3-4 mm segments, which were then transferred to MS medium supplemented with 0.5 mg/L BAP, 0.5 mg/L 2,4-D, 1 $\mu$g/L chlorsulfuron, 500 mg/L ticarcillin and 0.25% w/v

Gelrite. After 2 weeks, explants were transferred to fresh MS medium containing 0.16 mg/L thidiazuron (TDZ), 0.5 mg/L indolbutyric acid (IBA), 2 μg/L chlorsulfuron, 500 mg/L ticarcillin and 0.25% w/v Gelrite and care was taken at this stage to remove axillary shoots from stem explants. After 3 weeks, healthy adventitious shoots were transferred to hormone-free MS medium containing 3% w/v sucrose, 3 μg/L chlorsulfuron, 500 mg/L ticarcillin, 0.25% w/v Gelrite. Shoots which survived 3 μg/L chlorsulfuron were transferred to MS medium supplemented with 3% w/v sucrose, 500 mg/L ticarcillin, 5 μg/L chlorsulfuron and 0.25% w/v Gelrite for shoot elongation.

After 2-3 weeks, leaves were pulled from the shoots which had survived selection and were placed on a regeneration medium consisting of MS medium supplemented with 0.22 mg/L TDZ, 0.5 mg/L IBA, 3 μg/L chlorsulfuron, 500 mg/L ticarcillin and 0.25% w/v Gelrite, to obtain shoot regeneration in the presence of selection. Regenerated shoots were transferred to hormone-free MS medium containing 5μg/L chlorsulfuron, 500 mg/L ticarcillin and 0.25% w/v Gelrite for 2-4 weeks, then to hormone-free MS medium containing 200 mg/L ticarcillin and 0.4% w/v Gelrite, in glass jars, for normalization. Suncaps (Sigma) were placed on top of the glass jars to speed up the normalization of shoots. All cultures were maintained under a 16 h photoperiod (120 $\mu E/m^2/s$ cool white fluorescent light) at 23 ± 2°C. Normalized shoots, approximately 1.5-2 cm tall, were rooted on 3 g/kg IBA rooting powder and acclimatised under mist. A soil mix containing 75% perlite/25% peat was used for acclimation, which was carried out at 23°C under a 14 hour photoperiod (200 $\mu E/m^2/s$ mercury halide light) and typically lasted 3-4 weeks. Plants were fertilized with a carnation mix containing 1g/L CaNO, and 0.75 g/L of a mixture of microelements plus N:P:K in the ratio 4.7:3.5: 29.2.

For selection of transformed leaf tissue, leaves were transferred to a fresh medium consisting of MS medium supplemented with B5 vitamins; 590 mg/L MES: 0.5 mg/L BAP; 0.5 mg/L 2,4-D; 30g/L sucrose; 0.25 % w/v Gelrite; 500 mg/L carbenicillin and 2 μg/L chlorsulfuron, pH 5.8, for 2 weeks. Leaf explants were then transferred to a regeneration medium consisting of MS salts supplemented with B5 vitamin; 590 mg/L MES 0.5 mg/L IBA; 0.22

mg/L TDZ; 30g/L sucrose; 0.25% w/v Gelrite; 500 mg/L carbenicillin and 3 μg/L chlorsulfuron, pH 5.8. If small shoot clusters had formed after 2-3 weeks, they were separated into 2-4 sections. After another three weeks, regenerated shoots were harvested; leaves of the regenerated shoots were pulled apart and plated on fresh regeneration medium to undergo secondary regeneration. Transformed, vitrified shoots regenerated from the leaves within three weeks. To normalize, they were transferred to hormone-free MS medium containing 1% TC agar and 3μg/L chlorsulfuron and cultured for three weeks in plates and for an additional three weeks in Magenta™ GA-7 cubes. Within 2-3 weeks normal shoots formed and were rooted in hormone-free MS medium containing 0.2% w/v Gelrite. Rooted plants were transferred to soil, hardened off gradually, and then transferred to greenhouse conditions.

## EXAMPLE 11

### Isolation of carnation ACC oxidase (ACO) clone from cv. Scania

*a. Preparation of $^{32}$P-labelled probes*

Twenty micrograms of total RNA was incubated at 100°C for 2 minutes and then cooled on ice for a further 2 minutes. The RNA was added to a reaction mixture containing 20μg/ml oligo-dT, 50mM Tris-HCl pH 8.0, 75mM KCl, 30mM $MgCl_2$, 10mM DTT, 0.5 mg/mL actinomycin D, 200μM dATP, 200μM dGTP, 200μM dTTP, 2.5μM dCTP, 100μCi [α-$^{32}$P]-dCTP (Bresatec, 3000Ci/mmol), 40 units ribonuclease inhibitor (Promega), and 600 units MMLV reverse transcriptase (BRL) and incubated for 1 hour at 37°C. EDTA and NaOH were added to a final concentration of 50mM and 0.2M, respectively and the mixture was incubated for 20 minutes at 70°C. The mixture was then neutralised by addition of HCl to a concentration of 0.2M. Unincorporated [α-$^{32}$P]-dCTP was removed by chromatography on a Sephadex G-50 (Fine) column.

*b. $^{32}$P-Labelling of DNA fragments*

DNA fragments (50 to 100 ng) were radioactively labelled with 50 μCi of [α-$^{32}$P]-dCTP using an oligolabelling kit (Bresatec). Unincorporated [α-$^{32}$P]-dCTP was removed by chromatography on a Sephadex G-50 (Fine) column.

c. *Differential Screening of carnation cv. Scania cDNA library*

A cDNA library was constructed using mRNA from senescing carnation petals of the cv. Scania and the Lambda ZAP cDNA cloning vector (Stratagene), as described in Example 6c, above. A differential screening approach was used to isolate cDNA clones representing genes expressed in senescing carnation petals but reduced in flowers at the time of harvest. Thirty thousand colonies were screened at 1,500 colonies per 15cm plate. Duplicate plaque lifts were hybridized with cDNA probes from either (i) day 0 petal or (ii) in rolling petal and washed under high stringency conditions: hybridization on nitrocellulose in 50% v/v formamide, 6 x SSC, 1% w/v SDS at 42°C for 16 h and washing in 0.2 x SSC, 1% w/v SDS at 65°C for 3 x 30 min. Filters were then exposed to Kodak XAR film with an intensifying screen at -70°C for 16 hours. Clones which hybridized with the in rolling petal cDNA, but not with the day 0 cDNA, were selected for further investigation.

## EXAMPLE 12

### Sequence analysis of carnation cv. Scania ACO cDNA clone

Several senescence-associated cDNA clones were identified. The DNA sequence of one of the clones, a 1,156 bp sequence designated pCGP363, had 68% homology to the DNA sequence of a tomato cDNA clone, pTOM13, associated with ethylene production and fruit ripening. Later, pTOM13 was identified as encoding ACC oxidase (Hamilton *et al.*, 1991; Holdsworth *et al.*, 1987; Spanu *et al.*, 1991). The deduced amino acid sequence of 321 amino acids shares 68% identity with the tomato ACO amino acid sequence (Holdsworth et al, 1987), 74.6% identity with apple ACO (Dong *et al.*, 1992) and greater than 99% identity with the ACO sequence from another cultivar of carnation, White Sim (Wang and Woodson, 1991). The Scania sequence differs from that of White Sim only at amino acid residue 147. An alanine in the White Sim sequence is replaced by a glycine in the Scania sequence.

DNA sequencing of this and other clones was performed essentially by the method of Sanger *et al.* (1977) using the Sequenase enzyme (USB, version 2.1). The 1,156 bp carnation cv. Scania ACO sequence is presented as SEQ ID NO:7.

Homology searches against Genbank, SWISS-PROT and EMBL databases were again performed using the FASTA and LFASTA programmes (Pearson and Lipman, 1988). Alignment and comparison of the carnation cv. Scania ACC oxidase sequence with eight other sequences as follows: carnation cv. White Sim; *Arabidopsis thaliana;* tomato; orchid; apple; petunia; sunflower and geranium, can be seen in Figure 3. Alignments were performed using the Clustal V programme (Higgins *et al.*, 1991). Percentage similarities ranged from 95%, between carnation cultivars, to 72 % between carnation and for geranium.

## EXAMPLE 13

### Construction of pCGP 407

Vector pCGP407 was constructed using the standard techniques described in Sambrook *et al.* (1989). The carnation ACO cDNA fragment, contained within pCGP363 (see Example 12), was inserted in reverse orientation into a binary expression vector, pCGP293 (Brugliera *et al.*, 1994), between the MAC promoter (Comai *et al.*, 1990) and the mas 3' terminator region (from the *Agrobacterium* mannopine synthase gene). According to Comai *et al.* (1990), MAC is a strong constitutive promoter. The binary vector pCGP407 contained the neomycin phosphotransferase (NPT II) gene, in addition to the antisense ACO nucleic acid molecule, allowing selection of transgenic shoots by growth on kanamycin (Figure 4).

## EXAMPLE 14

### Transformation of *E. coli* and *A. tumefaciens* with pCGP407

Transformation of the *Escherichia coli* strain XL1-Blue with the vector pCGP407 was performed according to standard procedures (Sambrook *et al.*, 1989) or Inoue *et al.*, (1990).

The plasmid pCGP407 was introduced into *Agrobacterium tumefaciens* strain AGL0 by adding 5 $\mu$g of plasmid DNA to 100 $\mu$L of competent *Agrobacterium tumefaciens* cells prepared by inoculating a 50 mL MG/L (Garfinkel and Nester, 1980) culture and growing for 16 h with shaking at 28°C. The cells were then pelleted and resuspended in 0.5 mL of 85% v/v 100 mM $CaCl_2$/15% v/v glycerol. The DNA-*Agrobacterium* mixture was frozen by incubation in liquid $N_2$ for 2 min and then allowed to thaw by incubation at 37°C for 5

min. The DNA/bacterial mixture was then placed on ice for a further 10 min. The cells were then mixed with 1 mL of MG/L media and incubated with shaking for 16 h at 28°C. Cells of *A. tumefaciens* carrying pCGP407 were selected on MG/L agar plates containing 100 μg/mL gentamycin. The presence of the plasmid was confirmed by Southern analysis of DNA isolated from the gentamycin-resistant transformants.

## EXAMPLE 15

### Transformation of *Dianthus caryophyllus* with ACC oxidase

*a. Plant Material*

*Dianthus caryophyllus* (cvs. White Sim and Scania) cuttings were obtained from Van Wyk and Son Flower Supply, Victoria, Australia. The outer leaves were removed and the cuttings were sterilized briefly in 70% v/v ethanol followed by 1.25% w/v sodium hypochlorite (with Tween 20) for 6 minutes and rinsed three times with sterile water. All the visible leaves and axillary buds were removed under the dissecting microscope before co-cultivation.

*b. Co-cultivation of Agrobacterium and Dianthus Tissue*

*Agrobacterium tumefaciens* strain AGL0 (Lazo *et al.*, 1991), containing the binary vector pCGP407, was maintained at 4°C on LB agar plates with 50 mg/L tetracycline. A single colony was grown overnight in liquid LB broth containing 50 mg/L tetracycline. The following day it was diluted to 5 x $10^8$ cells/mL with liquid MS medium, before inoculation. *Dianthus* stem tissue was co-cultivated with *Agrobacterium* for 5 days on MS medium supplemented with 3% w/v sucrose, 0.5 mg/L BAP, 0.5 mg/L 2,4-D, 100 μM acetosyringone and 0.25% w/v Gelrite (pH 5.7).

*c. Recovery of Transgenic Dianthus Plants*

For selection of transformed stem tissue, the top 6-8 mm of each co-cultivated stem was cut into 3-4 mm segments, which were then transferred to MS medium supplemented with 1 mg/L BAP, 0.1 mg/L NAA, 150 mg/L kanamycin, 500 mg/L ticarcillin and 0.8% Difco Bacto Agar (selection medium). After three weeks, explants were transferred to fresh

selection medium and care was taken at this stage to remove axillary shoots from stem explants. After 6-8 weeks on selection medium healthy adventitious shoots were transferred to hormone-free MS medium containing 3% w/v sucrose, 150 mg/L kanamycin, 500 mg/L ticarcillin, 0.8% Difco Bacto Agar. At this stage, NPT II dot-blot assay (McDonnell *et al.*, 1987) was used to identify transgenic shoots. Transgenic shoots were transferred to MS medium supplemented with 3% w/v sucrose, 500 mg/L ticarcillin and 0.4% w/v Gelrite for shoot elongation. All cultures were maintained under a 16 hour photoperiod (120 $\mu E/m^2/s$ cool white fluorescent light) at 23 ± 2°C. When plants were rooted and reached 4-6 cm tall they were acclimatised under mist. A mix containing a high ratio of perlite (75% or greater) soaked in hydroponic mix (Kandreck and Black, 1984) was used for acclimation, which typically lasted 4-5 weeks. Plants were acclimatised at 23°C under a 14-hour photoperiod (200 $\mu E/m^2/s$ mercury halide light).

## EXAMPLE 16

### Southern Analysis

*a. Isolation of Genomic DNA from Dianthus*

DNA was isolated from tissue essentially as described by Dellaporta *et al.*, (1983). The DNA preparations were further purified by CsCl buoyant density centrifugation (Sambrook *et al.*, 1989).

*b. Southern Blots*

The genomic DNA (10 $\mu g$) was digested with *Eco*RI (for sense ACS) or *Hin*dIII (for antisense ACO) and electrophoresed through a 0.7% w/v or 0.8% w/v, respectively, agarose gel in a running buffer of TAE (40 mM Tris-acetate, 50 mM EDTA). The DNA was then denatured in denaturing solution (1.5 M NaCl/0.5 M NaOH) for 1 to 1.5 hours, neutralized in 0.5 M Tris-HCl (pH 7.5)/ 1.5 M NaCl for 2 to 3 hours and the DNA was then transferred to a Hybond N (Amersham) filter by capillary transfer (Sambrook *et al.*, 1989) in 20 x SSC.

Southern analysis of putative transgenic *Dianthus* plants obtained after selection on either chlorsulfuron or kanamycin confirmed the integration of the appropriate chimaeric gene into the genome, as shown in Figures 5 and 6.

## EXAMPLE 17

### Northern Analysis

Total RNA was isolated from tissue that had been frozen in liquid $N_2$ and ground to a fine powder using a mortar and pestle. An extraction buffer of 4 M guanidinium isothiocyanate, 50 mM Tris-HCl (pH 8.0), 20 mM EDTA, 0.1% v/v Sarkosyl, was added to the tissue and the mixture was homogenized for 1 minute using a polytron at maximum speed. The suspension was filtered through Miracloth (Calbiochem) and centrifuged in a JA20 rotor for 10 minutes at 10,000 rpm. The supernatant was collected and made to 0.2 g/ mL CsCl w/v. Samples were then layered over a 10 mL cushion of 5.7 M CsCl, 50 mM EDTA (pH 7.0) in 38.5 mL Quick-seal centrifuge tubes (Beckman) and centrifuged at 42,000 rpm for 12-16 hours at 23°C in a Ti-70 rotor. Pellets were resuspended in TE/SDS (10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.1% w/v SDS) and extracted with phenol:chloroform:isoamyl alcohol (25:24:1) saturated in 10 mM EDTA (pH 7.5). The RNA was then maintained as an ethanol precipitate, and appropriate aliquots pelleted prior to use.

RNA samples were electrophoresed through 2.2 M formaldehyde/1.2% w/v agarose gels using running buffer containing 40 mM morpholino-propanesulphonic acid (pH 7.0), 5 mM sodium acetate, 0.1 mM EDTA (pH 8.0). The RNA was transferred to Hybond-N filters (Amersham) as described by the manufacturer and probed with $^{32}$P-labelled cDNA fragment ($10^8$ cpm/$\mu$g, 2 x $10^6$ cpm/mL). Prehybridization (1 h at 42°C) and hybridization (16 h at 42°C) was carried out in 50% v/v formamide, 1 M NaCl, 1% w/v SDS, 10% w/v dextran sulphate, 100 $\mu$g/mL salmon sperm DNA.

Filters were washed in 2 x SSC/1% w/v SDS at 65°C for 1 hour and then 0.2 x SSC/1% w/v SDS at 65°C for 1 hour. In the case of antisense ACO, however, filters were also washed in 0.1 x SSC/0.1% w/v SDS at 65°C for 1 hour. All filters were exposed to Kodak XAR film

with an intensifying screen at -70°C for 48 hours.

Northern analysis of sense ACS plants indicated that the ALS transgene was expressed in the leaves of six of the eight lines assayed (see Figure 7).

Northern analysis of antisense ACO plants indicated that petals from transgenic Scania and White Sim flowers produce only very low levels of ACO and ACS mRNA at days 4 to 6, the time when inrolling would occur in normal, control flowers (see Figure 8).

## EXAMPLE 18

### $^{32}$P-Labelling of DNA Probes

DNA fragments (50 to 100 ng) were radioactively labelled with 50 $\mu$Ci of [$\alpha$-$^{32}$P]-dCTP using an oligolabelling kit (Bresatec). Unincorporated [$\alpha$-$^{32}$P]-dCTP was removed by chromatography on a Sephadex G-50 (Fine) column.

## EXAMPLE 19

### Transformation of *Dianthus* cultivars

The genetic contructs contained in the plasmids pWTT2160 and pCGP407 were introduced into various varieties of carnation using *Agrobacterium*-mediated gene transfer, as described in Examples 10 and 15, above. Integration of the appropriate DNA into the plant genome was confirmed by Southern analysis of plants obtained after kanamycin or chlorsulfuron selection, as described in Example 16.

Plants successfully rendered transgenic, in accordance with the present invention, have significantly reduced levels of climacteric ethylene production, compared with non-transgenic controls. For example, measurements of ethylene production, using a Varian model 3300 gas chromatograph equipped with a Porapak® N column (80C), flame ionization detector and Varian 4400 Integrator, indicated that flowers of carnation cvs. Scania and White Sim carrying the introduced antisense ACO genetic construct had a greatly reduced capacity to produce ethylene. The graph in Figure 9 shows ethylene evolution by transgenic

and control (non-transgenic) flowers from day of harvest onwards. Control plants produced flowers which synthesized normal amounts of ethylene, showing the expected climacteric rise in ethylene production at the onset of inrolling. Transgenic flowers of carnation cvs. Scania and White Sim produced less than 10% of the level of ethylene produced by control flowers.

## EXAMPLE 20

### Prolonged post-harvest survival

The introduction of one or more additional copies of either the ACC synthase or ACC oxidase DNA sequences into a plant's genome is capable of having a marked effect on the post-harvest life of the cut-flower. It has been possible to suppress the expression of the endogenous gene, using either a sense transcript and the co-suppression technology disclosed in US Patent Numbers 5,034,323; 5,231,020 and 5,238,184, or an antisense transcript and the antisense technology disclosed in US Patent Number 5,107,065, thereby generating transformed carnation flowers which produce significantly reduced levels of climacteric ethylene. These flowers exhibit post-harvest survival times often in excess of twice the normal vase-life of their non-transformed equivalents, and in the absence of the usual treatment with chemicals such as the environmentally-toxic silver thiosulphate. Exemplification of the "long-life" phenotype, using the sense ACS approach, is shown in Figures 10(A)-10(F), 11(A)-11(F), 12(A)-12(F), and 13(A)-13(D).

All flowers were kept in water and under 12h day/night cycle in controlled conditions, (1000 lux, 22°C, 65% relative humidity) following harvest. Figure 10(A)-10(F) shows transgenic carnation flowers of the cultivar Scania at 0, 4, and 11 days post-harvest. Control non-transgenic flowers are shown at 0, 4 and 7 days post-harvest. The transgenic flower still looks fresh at 11 days, while the non-transgenic equivalent already shows petal in-rolling, typical of senescing carnation flowers, at 4 days post-harvest and is totally senesced by 7 days post-harvest. Comparable results have been obtained for the cultivars Red Corso; Ember Rose and Crowley Sim, as seen in Figures 11(A)-11(F), 12(A)-12(F), and 13(A)-13(D), respectively. In each case, the transgenic carnation flower appears fresher for longer, when

compared with the non-transgenic control.

Transgenic, "long-life" flowers of the carnation cv. White Sim have also been produced using the sense ACS approach, in accordance with the present invention, as may be seen in Figure 14(A)-14(C). The non-transgenic control White Sim flower (on the left in each photograph) has begun to inroll and senesce by 11 days post-harvest and is completely senesced at 20 days post-harvest. By contrast, the three ACS sense-suppressed transgenic flowers appear as fresh as new at 11 days post-harvest and are still not in-rolling at 20 days post-harvest.

Furthermore, flowers from plants rendered transgenic using antisense ACO have also been produced for the carnation cultivars White Sim and Scania. The level of ACO mRNA has been suppressed and, hence, climacteric ethylene production all but eliminated and carnation flower vase life correspondingly extended. The normal vase life of these flowers is approximately 5 days from day of harvest to the beginning of inrolling. Flowers from transgenic Scania and White Sim had a vase life of 8 to 9 days, after which the petals slowly discoloured and dessicated without displaying the inrolling behaviour typical of carnation flower senescence. All control plants produced flowers of normal senescence phenotype. A transgenic, "long-life" flower of Scania, compared with a non-transgenic control flower at 6 days post-harvest, can be seen in Figure 15. Figure 16 shows a photograph of a transgenic, "long-life" White Sim flower next to a flower from a non-transgenic White Sim control plant, both at 8 days post-harvest. The transgenic flower still appears fresh while the control non-transgenic flower has completely senesced.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: ALLRAD NO.1 PTY LTD and
FLORIGENE INVESTMENTS PTY LTD

(ii) TITLE OF INVENTION: TRANSGENIC CARNATIONS EXHIBIT
PROLONGED POST-HARVEST LIFE

(iii) NUMBER OF SEQUENCES: 7

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: DAVIES COLLISON CAVE
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT INTERNATIONAL
(B) FILING DATE: 09-MAY-1996

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: PN2862 (AU)
(B) FILING DATE: 09-MAY-1995

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGGGT(C/T)TNG CNGAAAATCA GC 22

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

A(G/A)CANACNCG (A/G)AACCANCCN GG 22

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1942 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 134..1684

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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TATATATATA TACCCTCCAT TTTTCCTACT CCCCCTCCAC AAAAAATATA ATAATAGTGA 120

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CTT TCA AAA ATC GCT ACG AAC GAT GGC CAT GGT GAG AAT TTG GAG TAC          217
Leu Ser Lys Ile Ala Thr Asn Asp Gly His Gly Glu Asn Leu Glu Tyr
        15                  20                  25

TTT GAT GGG TGG AAA GCT TAT GAT AGA GAT CCT TAT CAT TCT ACC AAG          265
Phe Asp Gly Trp Lys Ala Tyr Asp Arg Asp Pro Tyr His Ser Thr Lys
     30                  35                  40

AAT TCT AAT GGC GTT ATT CAA ATG GGT CTC GCT GAA AAT CAG CTT TGC          313
Asn Ser Asn Gly Val Ile Gln Met Gly Leu Ala Glu Asn Gln Leu Cys
 45                  50                  55                  60

TTC GAT TTA GTT ACG GAG TGG CTA CTC AAA AAC CCA CAA GCC TCA ATT          361
Phe Asp Leu Val Thr Glu Trp Leu Leu Lys Asn Pro Gln Ala Ser Ile
                 65                  70                  75

TGT ACC AAC GAA GGT GTA AAT AAG TTC ATG GAT ATT GCC ATT TTT CAG          409
Cys Thr Asn Glu Gly Val Asn Lys Phe Met Asp Ile Ala Ile Phe Gln
             80                  85                  90

GAT TAT CAT GGT TTG CCC GAG TTT AGA AGT GCT GTG GCA AAA TTT ATG          457
Asp Tyr His Gly Leu Pro Glu Phe Arg Ser Ala Val Ala Lys Phe Met
         95                  100                 105

GGG AAG GCA AGA GAT GAG AAA GTC ATA TTC AAT CCA GAT AGA ATT GTA          505
Gly Lys Ala Arg Asp Glu Lys Val Ile Phe Asn Pro Asp Arg Ile Val
    110                 115                 120

ATG AGT GGT GGA GCC AGT GCA AGT GAA ACT CTT TTG TTT TGC TTG GCC          553
Met Ser Gly Gly Ala Ser Ala Ser Glu Thr Leu Leu Phe Cys Leu Ala
125                 130                 135                 140

AAC CCC GGT GAC GCC TTT TTA ATT CCG TCT CCT TAT TAT CCC GCA TTT          601
Asn Pro Gly Asp Ala Phe Leu Ile Pro Ser Pro Tyr Tyr Pro Ala Phe
                145                 150                 155

AAC CGC GAT TTA CGG TGG AGA ACT GGA GTA AAT TTA ATC CCA TTT ACT          649
Asn Arg Asp Leu Arg Trp Arg Thr Gly Val Asn Leu Ile Pro Phe Thr
            160                 165                 170

TGC TCG AGC TCG AAT AAT TTC AAA ATC ACT AAG GAA GCC TTA CAA TCG          697
Cys Ser Ser Ser Asn Asn Phe Lys Ile Thr Lys Glu Ala Leu Gln Ser
        175                 180                 185

GCA TAT GAA GAC GCC CTT AAA AAG AAC ATC AAA GTT AAG GGT ATT ATC          745
Ala Tyr Glu Asp Ala Leu Lys Lys Asn Ile Lys Val Lys Gly Ile Ile
    190                 195                 200

GTC ACA AAC CCG TCA AAT CCC TTA GGA ACG GTC CTA GAC AAG GAC ACC          793
Val Thr Asn Pro Ser Asn Pro Leu Gly Thr Val Leu Asp Lys Asp Thr
205                 210                 215                 220
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CTA AAA ATG TTA TTA ACA TTC GTA AAT GCG AAA AAT ATA CAC CTT GTG        841
Leu Lys Met Leu Leu Thr Phe Val Asn Ala Lys Asn Ile His Leu Val
                225                 230                 235

TGT GAC GAG ATA TAT GCA ACC ACA GTA TTT AAT TCG CCG AGC TTT ATA        889
Cys Asp Glu Ile Tyr Ala Thr Thr Val Phe Asn Ser Pro Ser Phe Ile
            240                 245                 250

AGT GTT GCT GAG GTT ATA AAG GAC ATG CCT CAT GTA AAT CAA GAC CTT        937
Ser Val Ala Glu Val Ile Lys Asp Met Pro His Val Asn Gln Asp Leu
        255                 260                 265

GTT CAT ATT TTA TAT AGT TTG TCC AAG GAC ATG GGC ATG CCG GGC TTT        985
Val His Ile Leu Tyr Ser Leu Ser Lys Asp Met Gly Met Pro Gly Phe
    270                 275                 280

AGG GTT GGG ATC ATT TAC TCT TAT AAT GAC CGT GTC GTC TCA ACT GCT       1033
Arg Val Gly Ile Ile Tyr Ser Tyr Asn Asp Arg Val Val Ser Thr Ala
285                 290                 295                 300

CGT CGA ATG TCG AGT TTT GGA CTT GTT TCT TCT CAA ACT CAG TTT ATG       1081
Arg Arg Met Ser Ser Phe Gly Leu Val Ser Ser Gln Thr Gln Phe Met
                305                 310                 315

ATC GCG GCA TTG CTC TCA GAT GAT GAT TTT GTT AGA CGA TTC TTG GTT       1129
Ile Ala Ala Leu Leu Ser Asp Asp Asp Phe Val Arg Arg Phe Leu Val
            320                 325                 330

GAG AGT AGA GAC AGA CTC TTT CGA AGG CAC CAG CAT TTC ACA AGC GAG       1177
Glu Ser Arg Asp Arg Leu Phe Arg Arg His Gln His Phe Thr Ser Glu
        335                 340                 345

CTG GCT AAG ATA GGA ATA GGA TGC CTC CAA GGA AAC GCG GCA TTG TTT       1225
Leu Ala Lys Ile Gly Ile Gly Cys Leu Gln Gly Asn Ala Ala Leu Phe
    350                 355                 360

GTT TGG ATG GAT TTG AGG CAT CTA TTA GAC GAA GCA ACG GTT GAA AGA       1273
Val Trp Met Asp Leu Arg His Leu Leu Asp Glu Ala Thr Val Glu Arg
365                 370                 375                 380

GAG TTA AAG TTA TGG AGA GTG ATC ATC AAT GAA GTG AAA ATC AAT GTG       1321
Glu Leu Lys Leu Trp Arg Val Ile Ile Asn Glu Val Lys Ile Asn Val
                385                 390                 395

TCA CCG GGT TCG TCC TTC CTG TGC TCT GAG CCA GGG TGG TTT AGG GTT       1369
Ser Pro Gly Ser Ser Phe Leu Cys Ser Glu Pro Gly Trp Phe Arg Val
            400                 405                 410

TGC TTT GCC AAC ATG GAC AAT GCG ACC TTA GAC GTT GCA CTC AAT CGA       1417
Cys Phe Ala Asn Met Asp Asn Ala Thr Leu Asp Val Ala Leu Asn Arg
        415                 420                 425

ATT AGG TCT TTT GTA ACC CGT GGA AGG GTG GAC AAT TCA ACA ATG ACA       1465
Ile Arg Ser Phe Val Thr Arg Gly Arg Val Asp Asn Ser Thr Met Thr
    430                 435                 440
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ACA ACA TCA GCA AGA GCA GCA GCA ACA ACA ACA ACA ACA ACA ACA ACA      1513
Thr Thr Ser Ala Arg Ala Ala Ala Thr Thr Thr Thr Thr Thr Thr Thr
445                 450                 455                 460

ACA ACA ACA ACA ACA ACA ACA ACA ACG ACA ATT AAG AAG AAA CGA GGG      1561
Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Ile Lys Lys Lys Arg Gly
                465                 470                 475

CAA ATG GAG CTT CGA CTT AGC TTC AAC AAT CGA AGA TTC GAA GAC GGT      1609
Gln Met Glu Leu Arg Leu Ser Phe Asn Asn Arg Arg Phe Glu Asp Gly
            480                 485                 490

TTA ATG TCA CCT CAT AGC ATC CTA TTA TCT CCT CAC TCT CCT ATG CCT      1657
Leu Met Ser Pro His Ser Ile Leu Leu Ser Pro His Ser Pro Met Pro
        495                 500                 505

CAA TCA CCC CTT GTT AAA GCA AGA ACA TAAGTCTAAA ATCATGAGTT            1704
Gln Ser Pro Leu Val Lys Ala Arg Thr
    510                 515

ATTAATAATA AATTTATCGA ACCAGTGTGA CGCCATTGAA ACGGTGCGAC GGGAGTTGAA    1764

ACGGTGTGAA AGACCACATT CAGATGAAGC ATTATATCTT CTCAACAAAA CATTGAACTT    1824

AATATAATTC AATATAACTT CTCTGTAATT TCATGTATAC AAACACTATA AATATGTAGT    1884

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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1087 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1087

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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ATG GGT CTC GCT GAA AAT CAG CTT TGC TTC GAT TTA GTT ACG GAG TGG      48
Met Gly Leu Ala Glu Asn Gln Leu Cys Phe Asp Leu Val Thr Glu Trp
 1               5                  10                  15

CTA CTC AAA AAC CCA CAA GCC TCA ATT TGT ACC AAC GAA GGT GTA AAT      96
Leu Leu Lys Asn Pro Gln Ala Ser Ile Cys Thr Asn Glu Gly Val Asn
            20                  25                  30
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AAG TTC ATG GAT ATT GCC ATT TTT CAG GAT TAT CAT GGT TTG CCC GAG          144
Lys Phe Met Asp Ile Ala Ile Phe Gln Asp Tyr His Gly Leu Pro Glu
          35                  40                  45

TTT AGA AGT GCT GTG GCA AAA TTT ATG GGG AAG GCA AGA GAT GAG AAA          192
Phe Arg Ser Ala Val Ala Lys Phe Met Gly Lys Ala Arg Asp Glu Lys
      50                  55                  60

GTC ATA TTC AAT CCA GAT AGA ATT GTA ATG AGT GGT GGA GCC AGT GCA          240
Val Ile Phe Asn Pro Asp Arg Ile Val Met Ser Gly Gly Ala Ser Ala
 65                  70                  75                  80

AGT GAA ACT CTT TTG TTT TGC TTG GCC AAC CCC GGT GAC GCC TTT TTA          288
Ser Glu Thr Leu Leu Phe Cys Leu Ala Asn Pro Gly Asp Ala Phe Leu
                 85                  90                  95

ATT CCG TCT CCT TAT TAT CCC GCA TTT AAC CGC GAT TTA CGG TGG AGA          336
Ile Pro Ser Pro Tyr Tyr Pro Ala Phe Asn Arg Asp Leu Arg Trp Arg
            100                 105                 110

ACT GGA GTA AAT TTA ATC CCA TTT ACT TGC TCG AGC TCG AAT AAT TTC          384
Thr Gly Val Asn Leu Ile Pro Phe Thr Cys Ser Ser Ser Asn Asn Phe
        115                 120                 125

AAA ATC ACT AAG GAA GCC TTA CAA TCG GCA TAT GAA GAC GCC CTT AAA          432
Lys Ile Thr Lys Glu Ala Leu Gln Ser Ala Tyr Glu Asp Ala Leu Lys
    130                 135                 140

AAG AAC ATC AAA GTT AAG GGT ATT ATC GTC ACA AAC CCG TCA AAT CCC          480
Lys Asn Ile Lys Val Lys Gly Ile Ile Val Thr Asn Pro Ser Asn Pro
145                 150                 155                 160

TTA GGA ACG GTC CTA GAC AAG GAC ACC CTA AAA ATG TTA TTA ACA TTC          528
Leu Gly Thr Val Leu Asp Lys Asp Thr Leu Lys Met Leu Leu Thr Phe
                165                 170                 175

GTA AAT GCG AAA AAT ATA CAC CTT GTG TGT GAC GAG ATA TAT GCA ACC          576
Val Asn Ala Lys Asn Ile His Leu Val Cys Asp Glu Ile Tyr Ala Thr
            180                 185                 190

ACA GTA TTT AAT TCG CCG AGC TTT ATA AGT GTT GCT GAG GTT ATA AAG          624
Thr Val Phe Asn Ser Pro Ser Phe Ile Ser Val Ala Glu Val Ile Lys
        195                 200                 205

GAC ATG CCT CAT GTA AAT CAA GAC CTT GTT CAT ATT TTA TAT AGT TTG          672
Asp Met Pro His Val Asn Gln Asp Leu Val His Ile Leu Tyr Ser Leu
    210                 215                 220

TCC AAG GAC ATG GGC ATG CCG GGC TTT AGG GTT GGG ATC ATT TAC TCT          720
Ser Lys Asp Met Gly Met Pro Gly Phe Arg Val Gly Ile Ile Tyr Ser
225                 230                 235                 240

TAT AAT GAC CGT GTC GTC TCA ACT GCT CGT CGA ATG TCG AGT TTT GGA          768
Tyr Asn Asp Arg Val Val Ser Thr Ala Arg Arg Met Ser Ser Phe Gly
                245                 250                 255
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CTT GTT TCT TCT CAA ACT CAG TTT ATG ATC GCG GCA TTG CTC TCA GAT        816
Leu Val Ser Ser Gln Thr Gln Phe Met Ile Ala Ala Leu Leu Ser Asp
            260                 265                 270

GAT GAT TTT GTT AGA CGA TTC TTG GTT GAG AGT AGA GAC AGA CTC TTT        864
Asp Asp Phe Val Arg Arg Phe Leu Val Glu Ser Arg Asp Arg Leu Phe
        275                 280                 285

CGA AGG CAC CAG CAT TTC ACA AGC GAG CTG GCT AAG ATA GGA ATA GGA        912
Arg Arg His Gln His Phe Thr Ser Glu Leu Ala Lys Ile Gly Ile Gly
    290                 295                 300

TGC CTC CAA GGA AAC GCG GCA TTG TTT GTT TGG ATG GAT TTG AGG CAT        960
Cys Leu Gln Gly Asn Ala Ala Leu Phe Val Trp Met Asp Leu Arg His
305                 310                 315                 320

CTA TTA GAC GAA GCA ACG GTT GAA AGA GAG TTA AAG TTA TGG AGA GTG       1008
Leu Leu Asp Glu Ala Thr Val Glu Arg Glu Leu Lys Leu Trp Arg Val
                325                 330                 335

ATC ATC AAT GAA GTG AAA ATC AAT GTG TCA CCG GGT TCG TCC TTC CTG       1056
Ile Ile Asn Glu Val Lys Ile Asn Val Ser Pro Gly Ser Ser Phe Leu
            340                 345                 350

TGC TCT GAG CCA GGG TGG TTT AGG GTT TGC T                            1087
Cys Ser Glu Pro Gly Trp Phe Arg Val Cys
        355                 360
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CGGGATCCGC TACTAATGAA GAGCATGGC 29

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GCGGTACCAG GTGACGAAAG TGGTGACA 28

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1156 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 53..1015

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
AAAACAAATA CAAATACAAA TACAAATACA TTGAATTTGT TAATTAACGA AC ATG          55
                                                           Met
                                                            1

GCA AAC ATT GTC AAC TTC CCT ATC ATT GAC ATG GAG AAG CTC AAT AAT        103
Ala Asn Ile Val Asn Phe Pro Ile Ile Asp Met Glu Lys Leu Asn Asn
              5                  10                  15

TAT AAT GGT GTT GAG AGG AGT CTT GTT TTG GAC CAA ATT AAG GAT GCT        151
Tyr Asn Gly Val Glu Arg Ser Leu Val Leu Asp Gln Ile Lys Asp Ala
        20                  25                  30

TGT CAC AAC TGG GGA TTC TTC CAG GTG GTG AAC CAT AGT TTG TCA CAT        199
Cys His Asn Trp Gly Phe Phe Gln Val Val Asn His Ser Leu Ser His
     35                  40                  45

GAA CTG ATG GAC AAA GTG GAG AGG ATG ACA AAA GAG CAT TAC AAG AAA        247
Glu Leu Met Asp Lys Val Glu Arg Met Thr Lys Glu His Tyr Lys Lys
 50                  55                  60                  65
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TTC AGG GAG CAA AAG TTC AAA GAC ATG GTT CAG ACC AAA GGT TTA GTG          295
Phe Arg Glu Gln Lys Phe Lys Asp Met Val Gln Thr Lys Gly Leu Val
                    70                  75                  80

TCT GCT GAG TCT CAA GTC AAT GAC ATT GAT TGG GAG AGC ACC TTC TAC          343
Ser Ala Glu Ser Gln Val Asn Asp Ile Asp Trp Glu Ser Thr Phe Tyr
                85                  90                  95

CTT CGT CAT CGT CCC ACC TCC AAC ATC TCC GAG GTC CCT GAT CTC GAC          391
Leu Arg His Arg Pro Thr Ser Asn Ile Ser Glu Val Pro Asp Leu Asp
            100                 105                 110

GAC CAA TAC AGG AAG TTG ATG AAG GAG TTT GCA GCC CAG ATT GAG AGG          439
Asp Gln Tyr Arg Lys Leu Met Lys Glu Phe Ala Ala Gln Ile Glu Arg
        115                 120                 125

TTA TCC GAG CAA CTG TTG GAC TTG TTA TGT GAG AAC CTT GGC CTT GAG          487
Leu Ser Glu Gln Leu Leu Asp Leu Leu Cys Glu Asn Leu Gly Leu Glu
    130                 135                 140                 145

AAA GGC TAC CTT AAG AAT GCC TTC TAT GGT GCC AAT GGC CCC ACT TTT          535
Lys Gly Tyr Leu Lys Asn Ala Phe Tyr Gly Ala Asn Gly Pro Thr Phe
                150                 155                 160

GGT ACC AAG GTC AGC AAC TAC CCG CCT TGC CCC AAA CCC GAC CTT ATC          583
Gly Thr Lys Val Ser Asn Tyr Pro Pro Cys Pro Lys Pro Asp Leu Ile
            165                 170                 175

AAA GGA CTT AGG GCC CAC ACC GAC GCT GGT GGC ATC ATT CTC TTG TTC          631
Lys Gly Leu Arg Ala His Thr Asp Ala Gly Gly Ile Ile Leu Leu Phe
        180                 185                 190

CAG GAC GAC AAG GTC AGC GGC CTC CAG CTC CTC AAG GAT GGT CAT TGG          679
Gln Asp Asp Lys Val Ser Gly Leu Gln Leu Leu Lys Asp Gly His Trp
    195                 200                 205

GTT GAT GTT CCT CCC ATG AAA CAC TCC ATT GTT GTT AAC TTG GGG GAC          727
Val Asp Val Pro Pro Met Lys His Ser Ile Val Val Asn Leu Gly Asp
210                 215                 220                 225

CAA CTT GAG GTT ATT ACA AAT GGC AAG TAC AAG AGT GTG ATG CAC CGC          775
Gln Leu Glu Val Ile Thr Asn Gly Lys Tyr Lys Ser Val Met His Arg
                230                 235                 240

GTG ATA GCG CAG ACA GAT GGT AAC AGG ATG TCG ATA GCA TCA TTC TAC          823
Val Ile Ala Gln Thr Asp Gly Asn Arg Met Ser Ile Ala Ser Phe Tyr
            245                 250                 255

AAC CCG GGA AGT GAT GCC GTG ATT TAC CCG GCG CCA ACA TTG GTG GAA          871
Asn Pro Gly Ser Asp Ala Val Ile Tyr Pro Ala Pro Thr Leu Val Glu
        260                 265                 270

AAA GAA GAG GAG AAA TGC AGA GCA TAC CCA AAA TTT GTG TTC GAG GAT          919
Lys Glu Glu Glu Lys Cys Arg Ala Tyr Pro Lys Phe Val Phe Glu Asp
    275                 280                 285
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TAC ATG AAT CTC TAC TTA AAG CTC AAG TTC CAA GAG AAG GAG CCC AGG        967
Tyr Met Asn Leu Tyr Leu Lys Leu Lys Phe Gln Glu Lys Glu Pro Arg
290                 295                 300                 305

TTT GAA GCA ATG AAG GCC ATG GAA ACC ACG GGT CCC ATT CCA ACT GCT       1015
Phe Glu Ala Met Lys Ala Met Glu Thr Thr Gly Pro Ile Pro Thr Ala
                310                 315                 320

TGAAATAATG ATTTGATTTG ATATAATGCA ATGCTTCTCA TCAACCAATT TAAGTATTTC     1075

TAATATACGC CACTCTCATC TCATCTCATA TATTCATATT CATATTATTA GTGTTTGTTG     1135

AATAAGAGCT TCCTTTTAAG T                                              1156
```

CLAIMS:

1. A method for producing a transgenic plant exhibiting reduced production of climacteric ethylene, compared to its non-transgenic parent or a non-transgenic plant of the same species, said method comprising introducing into a cell or cells of a plant a genetic construct comprising a nucleic acid molecule encoding, or complementary to a sequence encoding ACC synthase or ACC oxidase or a derivative of said nucleic acid molecule, and regenerating a transgenic plant from said cell or cells.

2. A method according to claim 1 wherein the transgenic plant exhibits one or more of the following properties:

(i) a reduction in production of ACC synthase-specific mRNA or ACC oxidase-specific mRNA;

(ii) a reduction in production of ACC synthase or ACC oxidase; and/or

(iii) delayed senescence of flowers or flower buds cut from said transgenic plant.

3. A method according to claim 1 or 2 wherein the genetic construct comprises a non-full length fragment of a nucleic acid molecule encoding ACC synthase or ACC oxidase.

4. A method according to claim 3 wherein the non-full length fragment is approximately 800-1200 base pairs in length.

5. A method according to claim 3 wherein the non-full length fragment is an internal fragment of the nucleic acid molecule encoding ACC synthase or ACC oxidase.

6. A method according to claim 3 or 4 or 5 wherein reduction in production of ACC synthase-specific mRNA or ACC oxidase-specific mRNA or reduction in production of ACC synthase or ACC oxidase is achieved by co-suppression.

7. A method for producing a transgenic carnation plant having flowers or flower buds which, when cut from said carnation plant, exhibit prolonged post-harvest life properties relative to its non-transgenic parent or a non-transgenic plant of the same species, said method comprising introducing into a cell or cells of a plant a genetic construct comprising a non-full length fragment of a nucleic acid molecule encoding, or complementary to a sequence encoding, ACC synthase or ACC oxidase, and regenerating a plant from said cell or cells wherein flowers of the said transgenic plant exhibit one or more of the following properties:

(i) a reduced level of ACC synthase-specific mRNA or ACC oxidase-specific mRNA below non-transgenic endogenous levels:

(ii) a reduced level of ACC synthase or ACC oxidase below non-transgenic endogenous levels; and/or

(iii) a reduced level of climacteric ethylene production below non-transgenic endogenous levels.

8. A method according to claim 7 wherein the non-full length fragment of the nucleic acid molecule is approximately 800-1200 bp in length and the reduction in ACC synthase-specific mRNA or ACC oxidase-specific mRNA or, reduction in ACC synthase or ACC oxidase or reduction in climacteric ethylene production is by co-suppression.

9. A method according to claim 1 or 8 wherein the nucleic acid molecule comprises a sequence of nucleotides substantially as set forth in SEQ ID NO:3 or is a derivative thereof or is a nucleic acid molecule capable of hybridising to the sequence of nucleotides set forth in SEQ ID NO:3 under low stringency conditions at 30°C or is a nucleic acid molecule having a nucleotide sequence having at least about 60% similarity to the sequence of nucleotides set forth in SEQ ID NO:3.

10. A method according to claim 1 or 8 wherein the nucleic acid molecule comprises a sequence of nucleotides substantially as set forth in SEQ ID NO:4 or is a derivative thereof or is a nucleic acid molecule capable of hybridising to the sequence of nucleotides set forth in SEQ ID NO:4 under low stringency conditions at 30°C or is a nucleic acid molecule having a

nucleotide sequence having at least about 60% similarity to the sequence of nucleotides set forth in SEQ ID NO:4.

11. A method according to claim 1 or 8 wherein the nucleic acid molecule comprises a sequence of nucleotides substantially as set forth in SEQ ID NO:7 or is a derivative thereof or is a nucleic acid molecule capable of hybridising to the sequence of nucleotides set forth in SEQ ID NO:7 under low stringency conditions at 30°C or is a nucleic acid molecule having a nucleotide sequence having at least about 60% similarity to the sequence of nucleotides set forth in SEQ ID NO:7.

12. A method according to claim 1 or 8 wherein the nucleic acid molecule encodes an amino acid sequence substantially as set forth in SEQ ID NO:3 or having at least about 40% similarity thereto.

13. A method according to claim 1 or 8 wherein the nucleic acid molecule encodes an amino acid sequence substantially as set forth in SEQ ID NO:4 or having at least about 40% similarity thereto.

14. A method according to claim 1 or 8 wherein the nucleic acid molecule encodes an amino acid sequence substantially as set forth in SEQ ID NO:7 or having at least about 40% similarity thereto.

15. A method for producing a transgenic flowering carnation plant wherein the flowers exhibit reduced levels of ethylene production relative to levels in its non-transgenic parent plant or a non-transgenic plant of the same species, said method comprising introducing into a cell or cells of a carnation plant, a genetic construct comprising nucleic acid molecule encoding, or complementary to a sequence encoding, ACC synthase or ACC oxidase or a derivative of said nucleic acid molecule and regenerating a transgenic plant from the cell or cells.

16. A method according to claim 15 wherein the nucleic acid molecule comprises a sequence of nucleotides substantially as set forth in SEQ ID NO:3 or is a derivative thereof or is a nucleic acid molecule capable of hybridising to the sequence of nucleotides set forth in SEQ ID NO:3 under low stringency conditions at 30°C or is a nucleic acid molecule having a nucleotide sequence having at least about 60% similarity to the sequence of nucleotides set forth in SEQ ID NO:3.

17. A method according to claim 15 wherein the nucleic acid molecule comprises a sequence of nucleotides substantially as set forth in SEQ ID NO:4 or is a derivative thereof or is a nucleic acid molecule capable of hybridising to the sequence of nucleotides set forth in SEQ ID NO:4 under low stringency conditions at 30°C or is a nucleic acid molecule having a nucleotide sequence having at least about 60% similarity to the sequence of nucleotides set forth in SEQ ID NO:4.

18. A method according to claim 15 wherein the nucleic acid molecule comprises a sequence of nucleotides substantially as set forth in SEQ ID NO:7 or is a derivative thereof or is a nucleic acid molecule capable of hybridising to the sequence of nucleotides set forth in SEQ ID NO:7 under low stringency conditions at 30°C or is a nucleic acid molecule having a nucleotide sequence having at least about 60% similarity to the sequence of nucleotides set forth in SEQ ID NO:7.

19. A method according to claim 15 wherein the nucleic acid molecule encodes an amino acid sequence substantially as set forth in SEQ ID NO:3 or having at least about 40% similarity thereto.

20. A method according to claim 15 wherein the nucleic acid molecule encodes an amino acid sequence substantially as set forth in SEQ ID NO:4 or having at least about 40% similarity thereto.

21. A method according to claim 15 wherein the nucleic acid molecule encodes an amino acid sequence substantially as set forth in SEQ ID NO:7 or having at least about 40% similarity thereto.

22. A method according to claim 1 or 7 or 15 wherein the genetic construct is plasmid pWTT2160 or plasmid pCGP407 deposited with the Australian Government Analytical Laboratory under Accession Numbers N95/26121 and N95/26122, respectively.

23. A transgenic carnation plant comprising a nucleic acid molecule encoding, or complementary to a sequence encoding, ACC synthase or ACC oxidase or a derivative of said nucleic acid molecule wherein said transgenic plant exhibits one or more of the following properties:

(i) a reduction in the production of ACC synthase-specific mRNA;

(ii) a reduction in the production of ACC synthase enzyme;

(iii) a reduction in the production of climacteric ethylene; and/or

(iv) delayed senescence of flowers or flower buds cut from said transgenic plants.

24. A transgenic plant according to claim 23 wherein the nucleic acid molecule is a non-full length fragment of a nucleic acid molecule encoding ACC synthase or ACC oxidase..

25. A transgenic plant according to claim 24 wherein the non-full length fragment is approximately 800-1200 base pairs in length.

26. A transgenic plant according to claim 25 wherein the non-full length fragment is an internal fragment of the nucleic acid molecule encoding ACC synthase or ACC oxidase.

27. A transgenic carnation plant capable of carrying flowers or flower buds with prolonged post-harvest life properties relative to its non-transgenic parent or a non-transgenic part of the same species, said plant comprising a non-full length fragment of a nucleic acid molecule encoding, or complementary to a sequence encoding, a ACC synthase or ACC oxidase wherein

flowers or flower buds of said transgenic plant exhibit one or more of the following properties:

(i) a reduced level of ACC synthase-specific mRNA or ACC oxidase-specific mRNA below non-transgenic endogenous levels;

(ii) a reduced level of ACC synthase or ACC oxidase enzyme below non-transgenic endogenous levels; and/or

(iii) a reduced level of ethylene production below non-transgenic endogenous levels.

28. A transgenic plant according to claim 27 wherein the non-full length fragment of the nucleic acid molecule encoding ACC synthase or ACC oxidase is approximately 800-1200 bp in length and endogenous ACC synthase or ACC oxidase gene expression is reduced by co-suppression.

29. A method according to claim 23 or 27 wherein the nucleic acid molecule comprises a sequence of nucleotides substantially as set forth in SEQ ID NO:3 or is a derivative thereof or is a nucleic acid molecule capable of hybridising to the sequence of nucleotides set forth in SEQ ID NO:3 under low stringency conditions at 30°C or is a nucleic acid molecule having a nucleotide sequence having at least 60% similarity to the sequence of nucleotides set forth in SEQ ID NO:3.

30. A method according to claim 23 or 27 wherein the nucleic acid molecule comprises a sequence of nucleotides substantially as set forth in SEQ ID NO:4 or is a derivative thereof or is a nucleic acid molecule capable of hybridising to the sequence of nucleotides set forth in SEQ ID NO:4 under low stringency conditions at 30°C or is a nucleic acid molecule having a nucleotide sequence having at least 60% similarity to the sequence of nucleotides set forth in SEQ ID NO:4.

31. A method according to claim 23 or 27 wherein the nucleic acid molecule comprises a sequence of nucleotides substantially as set forth in SEQ ID NO:7 or is a derivative thereof or is a nucleic acid molecule capable of hybridising to the sequence of nucleotides set forth in SEQ ID NO:7 under low stringency conditions at 30°C or is a nucleic acid molecule having a

nucleotide sequence having at least 60% similarity to the sequence of nucleotides set forth in SEQ ID NO:7.

32. A transgenic plant according to claim 23 or 27 wherein the nucleic acid molecule encodes an amino acid sequence substantially as set forth in SEQ ID NO:3 or having at least about 40% similarity thereto.

33. A transgenic plant according to claim 23 or 27 wherein the nucleic acid molecule encodes an amino acid sequence substantially as set forth in SEQ ID NO:4 or having at least about 40% similarity thereto.

34. A transgenic plant according to claim 23 or 27 wherein the nucleic acid molecule encodes an amino acid sequence substantially as set forth in SEQ ID NO:7 or having at least about 40% similarity thereto.

35. A cut flower from a transgenic carnation according to any one of claims 23 to 34.

36. Seeds or other reproductive material from a transgenic carnation according to any one of claims 23 to 34.

1/40

| |
|---|
| 2/40 |
| 3/40 |
| 4/40 |
| 5/40 |
| 6/40 |
| 7/40 |
| 8/40 |
| 9/40 |
| 10/40 |
| 11/40 |
| 12/40 |
| 13/40 |
| 14/40 |
| 15/40 |

```
CARNATION       --GGTCTTA-ATCTTTGTCACTTTA--CAACAACATA--AATTATATTCT
CARNATION       --G-------------------------------------------
PETUNIA         --GAACA-ACACTAC----AATA-AATCAACTTTTCTATAGAGA-----G
TOMATO          CCAAACACATAATACTTTTAATACAATTAGTTATTTATTAGAAGTATTTA
ORCHID          --GA------ATTCCT-----TGTAGCCTTCTCCTTCTT-----TTTTCT
ARABIDOPSIS     -------------------------------------------------
ZUCCHINI        C-----A------ACTTTCAA--------------------------

CARNATION       CAATCATTTTCTCTATATATATATATATACCCTCCATTTT-----TCCTA
CARNATION       -------------------------------------------------
PETUNIA         AAGCA--GTACTCAC-ATTTTTATAC-------CAACTTTCACATTC---
TOMATO          AAGTAAAGCACTTGTGAGTTGTGTACATTTTATTAATCTTCATCTTCTTA
ORCHID          AAGT----CTTTCTCCAATTA-ATACTTGTC-TCAATCT----------A
ARABIDOPSIS     -------------ATGGGT----------------------------
ZUCCHINI        -------------ATGGG--------GTTTCATCA-------------A

CARNATION       CTCCCCCTCCACAAAAAATATAATAA-TAGTGAGT--GTGTAATCAAAAT
CARNATION       -----------------------------------TGTAATCAAAAT
PETUNIA         ---CTCTCCAATATTTA--TTCATCACTT-TAAACTCATT-------AAG
TOMATO          ATTCTCTTCAGTTTTTAATTTCTTCACTTCTAAACTCATTTAGTAAAAAA
ORCHID          ATCTCTGCTATCAGTAAGTAC--TAATT---AAAATCATGT--CTAAAAT
ARABIDOPSIS     -------------------------CTTCCGGG-----------AAAAA
ZUCCHINI        ATC-------------------------------------------G
```

FIGURE 1A

```
CARNATION   GGGTTCTTA--TAAGGGTGTTTACGACCGTGAAATTCTTTCAAAAATCGC
CARNATION   GGGTTCTTA--TAAGGGTGTTTACGACCGTGAAATTCTTTCAAAAATCGC
PETUNIA     ACAATGGGATTTGAGAGTGAGAAGAACAACTCAGTCTTGTCTAAGCTTGC
TOMATO      AAAATGGGATTTGAGATTGCAAAGACCAACTCAATCTTATCAAAATTGGC
ORCHID      G---------TTTGGGAA-----AGAG--GTGCCAT--TGTCAAAAATGGC
ARABIDOPSIS ATAAAGG----TG-----------------CAGTTTTGTCGAAGATAGC
ZUCCHINI    ACGAAAGGAACCAAG-----------------CTC--TTCTCTCGAAGATCGC
                                            *  * ** **  * **

CARNATION   TACGAACGATGGCCATGGTGAGAATTTGGAGTACTTTGATGGGTGGAAAG
CARNATION   TACGAACGATGGCCATGGTGAGAATTTGGAGTACTTTGATGGGTGGAAAG
PETUNIA     TACTAATGAAGAACATGGCGAAAACTCACCATATTTTGATGGATGGAAAG
TOMATO      TACTAATGAAGAGCATGGCGAAAACTCGCCATATTTTGATGGGTGGAAAG
ORCHID      GGTGTCTAAAGCTCATGGAGAGGGCTCTCCATACTTCGCTGGCTGGAAAG
ARABIDOPSIS GACTAACAATCAACACGGAGAGAACTCAGAGTACTTTGATGGATGGAAAG
ZUCCHINI    CCTCGACGATGGCCATGGCGAGAACTCCCCGTATTTCGATGGGTGGAAAG
                *    ** ** **    *    ** ** * *** *******

CARNATION   CTTATGATAGAGATCCTTATCATTCTACCAAGAATTCTAATGGCGTTATT
CARNATION   CTTATGATAGAGATCCTTATCATTCTACCAAGAATTCTAATGGCGTTATT
PETUNIA     CTTATGATAACGATCCTTTTCACCCTTTGAAGAACCCTAACGGGGTTATC
TOMATO      CATACGATAGTGATCCTTTCCACCCTCTAAAAAACCCCAACGGAGTTATC
ORCHID      CTTATGAAGAGAATCCTTATGATGTTGTAGGAAATCCTGATGGAGTTATT
ARABIDOPSIS CTTACGACAAAGATCCTTTTCATCTTTCCCGTAACCCCCCATGGGATCATC
ZUCCHINI    CTTACGATAACGATCCGTTTCACCCTGAGAATAATCCCTTTGGGTGTTATT
            * ** **    **** * *  *  *    **  *    **  * **
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FIGURE 1B

```
CARNATION   CAAATGGGTCTCGCTGAAAATCAGCTTTGCTTCGATTTAGTTACGGAGTG
CARNATION   CAAATGGGTCTCGCTGAAAATCAGCTTTGCTTCGATTTAGTTACGGAGTG
PETUNIA     CAAATGGGACTTGCTGAAAATCAGCTCTGTTTTGACTTGATTGAGGACTG
TOMATO      CAAATGGGTCTTGCTGAAAATCAGCTTTGTTTAGACTTGATAGAAGATTG
ORCHID      CAGATGGGCTTAGCTGAGAATCAGCTTTCTTTTGATCTGCTGGAAGAGTA
ARABIDOPSIS CAAATGGGTCTTGCAGAGAATCAGCTTTGCTTAGATTTGATCAAAGATTG
ZUCCHINI    CAAATGGGTTTAGCAGAAAATCAGCTTTCCTTTGATATGATTGTTGACTG
            ** ***** * ** ** ********* *  ** **  *  *    ** *

CARNATION   GCTACTCAAAAACCCACAAGCCTCAATTTGTACCAACGAAGGTGTAAATA
CARNATION   GCTACTCAAAAACCCACAAGCCTCAATTTGTACCAACGAAGGTGTAAATA
PETUNIA     GATTAAGAGAAACCCAAAAGCTTCCATTTGCACTACTGAAGGATCAAAT
TOMATO      GATTAAGAGAAACCCAAAAGGTTCAATTTG---TTCTGAAGGAATCAAAT
ORCHID      CCTGGAGCTGCACCCTGAAGCTTTTAGCTGGGCTTCTGACTCCTCTAGT-
ARABIDOPSIS GGTCAAAGAGAACCCAGAAGCTTCTATTTGCACCCTTGAAGGTATTCATC
ZUCCHINI    GATTAGAAAACACCCTGAAGCTTCGATTTGTACACCGGAAGGACTTGAGA
               *       ****  ***  *  *  **       **

CARNATION   AGTTCATGGATATTGCCATTTTTCAGGATTATCATGGTTTGCCCGAGTTT
CARNATION   AGTTCATGGATATTGCCATTTTTTCAGGATTATCATGGTTTGCCCGAGTTT
PETUNIA     CTTTTAGGGGCATTGCTAACTTCCAAGATTATCATGGTTTACCTGAATTC
TOMATO      CATTCAAGGCCATTGCCAACTTTCAAGATTATCATGGCTTGCCTGAATTC
ORCHID      --TTTAGAGAAAATGCTTTGTTTCAGGACTATCATGGCCTCCAAACTCTC
ARABIDOPSIS AGTTTAGCGACATCGCTAATTTCCAAGACTACCATGGTCTTAAGAAGTTT
ZUCCHINI    GATTCAAAGCATTGCCAACTTCCAAGATTACCACGGCTTACCAGAGTTT
               ** *    *   **    ** ** ** ** ** **    *
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FIGURE 1C

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CARNATION   AGAAGTGCTGTGGCAAAATTTATGGGGAAGGCAAGAGATGAGAAAGTCAT
CARNATION   AGAAGTGCTGTGGCAAAATTTATGGGGAAGGCAAGAGATGAGAAAGTCAT
PETUNIA     AGAAGAGCTATTGCAAAGTTCATGGAAAAAACAAGAGGGGGTAGGGTTAG
TOMATO      AGAAAAGCGATTGCGAAATTTATGGAGAAAACAAGAGGAGGAAGAGTTAG
ORCHID      AGACAGGCATTGGCTAGCTTTATGGAGAAAATCAGAGGTGGTCGATCAAA
ARABIDOPSIS AGACAGGCAATTGCACATTTCATGGGAAAAGCTAGAGGTGGAAGAGTGAC
ZUCCHINI    CGAAATGCAATTGCAAATTTTATGGGGAAAGTAAGAGGTGGGAGGGTAAA
              **   **  * **   ** ****  **    ****  *        *

CARNATION   ATTCAATCCAGATAGAATTGTAATGAGTGGTGGAGCCA---GTGCAAGTG
CARNATION   ATTCAATCCAGATAGAATTGTAATGAGTGGTGGAGCCACGGGTGCAAGTG
PETUNIA     CTTTGATCCAGACCGAGTAGTTATGGCCGGTGGTGCCACTGGAGCTAACG
TOMATO      ATTTGATCCAGAAAGAGTTGTTATGGCTGGTGGTGCCACTGGAGCTAATG
ORCHID      GTTCGATGCCAACCGCATCGTCCTCACCGCCGGCGCCACCGCCGCCAACG
ARABIDOPSIS TTTTGATCCGGAGAGGGTGGTTATGAGCGGAGGAGCCACCGGAGCCAATG
ZUCCHINI    ATTCGACCCGAGTCGGATTGTGATGGGTGGCGGTGCGACCGGAGCGAGCG
              **  *  *     *  *  **  *     *  ** ** *      ** * *

CARNATION   AAACTCTTTTGTTTTGCTTGGCCAACCCCGGTGACGCCTTTTTAATTCCG
CARNATION   AAACTCTTTTGTTTTGCTTGGCCAACCCCGGTGACGCCTTTTTAATTCCG
PETUNIA     AGACAATCATATTTTGCTTGGCTGATGCTGGCGATGCATTCTTAGTACCT
TOMATO      AGACAATTATATTTTGTTTGGCTGATCCTGGCGATGCATTTTTAGTACCT
ORCHID      AGATCCTTACATTTATCTTAGCCGACCGCGGCGATGCCTTACTTGTCCCA
ARABIDOPSIS AAACAATCATGTTCTGCCTTGCGGATCCCGGCGACGTTTTCCTCATTCCC
ZUCCHINI    AAACCGTCATCTTTTGTTTGGCGGATCCGGGGGGATGCTTTTTTGGTTCCT
            * *   *    **     * **   *    ** ** *  **  *  * **
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FIGURE 1D

```
CARNATION   TCTCCTTATTATCCCGCATTTAACCGCGATTTACGGTGGAGAACTGGAGT
CARNATION   TCTCCTTATTATCCCGCATTTAACCGCGATTTACGGTGGAGAACTGGAGT
PETUNIA     TCACCTTATTACCCAGCATTTAACAGAGACCTAAGATGGAGAACTGGGGT
TOMATO      TCACCATACTACCCAGCATTTAACAGAGATTTAAGATGGAGAACTGGAGT
ORCHID      ACTCCTTATTATCCAG----------------------------------
ARABIDOPSIS TCCCCGTACTATGCCGCATTTGATAGAGACTTGAGGTGGCGGACAGGTGT
ZUCCHINI    TCTCCATATTATGCAGGATTTGATCGAGACTTGAAATGGCGAACACGAGC
              * ** ** **  * *

CARNATION   AAATTTAATCCCCATTTACTTGCTCGAGCTCGAATAATTTCAAAATCACTA
CARNATION   AAATTTAATCCCCATTTACTTGCTCGAGCTCGAATAATTTCAAAATCACTA
PETUNIA     ACAACTCATTCCAATTCCTTGCGAGAGCTCCAACAGCTTCAAAATTACTA
TOMATO      ACAACTTATTCCAATTCACTGTGAGAGCTCCAATAATTTCAAAATTACTT
ORCHID      ----------------------GCTCCAATGGCTTCCAACTGACTC
ARABIDOPSIS CGAGATAATCCCGGTTCCTTGTTCAAGCTCCGACAATTTCAAATTAACCG
ZUCCHINI    ACAAATAATTCGGGTCCATTGCAACGGCTCGAATAACTTCCAAGTCACAA
                                ****  *    *** ** * **

CARNATION   AGGAAGCCTTACAATCGGCATATGAAGACGCCCTTAAAAGAACATCAAA
CARNATION   AGGAAGCCTTACAATCGGCATATGAAGACGCCCTTAAAAGAACATCAAA
PETUNIA     CAAAAGCTATGAAAGAAGCATATGAAAATGCCATAAAAGCAAACATCAGA
TOMATO      CAAAAGCAGTAAAAGAAGCATATGAAAATGCACACAAAAATCAAACATCAAA
ORCHID      TCTCCTCCCTCGAAAAAGCCTACGCTGAAGCCAAAGCTTCCAACTTTAAT
ARABIDOPSIS TTGACGCCGCGGAATGGGCTTATAAAAAAGCCCAAGAGTCCAATAAAAAA
ZUCCHINI    AGGCAGCCTTAGAAATAGCCTACAAAAAGGCTCAAGAGGCCAACATGAAA
              *    **  ** **   * **      **    *
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FIGURE 1E

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CARNATION   GTTAAGGGTATTATCGTCACAAACCCGTCAAATCCCTTAGGAACGGTCCT
CARNATION   GTTAAGGGTATTATCGTCACAAACCCGTCAAATCCCTTAGGAACGGTCCT
PETUNIA     GTGAAAGGCTTGATTTTGACTAATCCATCAAATCCATTGGGCACCACTTT
TOMATO      GTAAAAGGTTTGATTTTGACCAATCCATCAAATCCATTGGGCACCACTTT
ORCHID      GTCAGGGGTCTTCTGATGACCAATCCTTGTAATCCTCTGGGCACCTCTGC
ARABIDOPSIS GTCAAAGGTCTGATTTTGACCAACCCATCAAATCCACTCGGTACAATGTT
ZUCCHINI    GTGAAGGGTGTTATAATCACCAATCCCTCAAATCCCTTAGGCACAACGTA
            ** *  **  *  *  * ** ** ** *  *****  * ** **

CARNATION   AGACAAGGACACCCTAAAAATGTTATTAACATTCGTAAATGCGAAAAATA
CARNATION   AGACAAGGACACCCTAAAAATGTTATTAACATTCGTAAATGCGAAAAATA
PETUNIA     GGACAGAGACACATTAAAAAGTCTCTTGAACTTCACCAACGAACGAAACA
TOMATO      GGACAAAGACACACTGAAAAGTGTCTTGAGTTTCACCAACCAACACAACA
ORCHID      CTCTCTTTCTCTTCTCCAAGACATAATTCACTTCATCTCAGACAAAAACA
ARABIDOPSIS GGATAAGGACACACTCACGAACTTGGTCCGGTTTGTCACGAGGAAGAACA
ZUCCHINI    CGACCGTGACACTCTTAAAACCCTCGTCACCTTTGTGAATCAACACGACA
                                 *  *  **     * *

CARNATION   TACACCTTGTGTGTGACGAGATATATGCAACCACAGTATTTAATTCGCCG
CARNATION   TACACCTTGTGTGTGACGAGATATATGCAACCACAGTATTTAATTCGCCG
PETUNIA     TCCACCTCGTTTGCGACGAAATTTATGCTGCCACTGTCTTTAACACACCA
TOMATO      TCCACCTTGTTTGTGACGAAATCTACGCAGCCACTGTCTTTGACACGCCT
ORCHID      TTCATCTGATCTCCGATGAGATCTACTCTGGCTCTGTTTTCTCTTCTACA
ARABIDOPSIS TTCACCTAGTCGTCGACGAGATCTACGCCGCCACAGTCTTCGCCGGAGGA
ZUCCHINI    TTCACTTAATATGCGATGAAATATACTCTGCCACTGTCTTCAAAGCCCCA
            * **  *  *    ** ** ** **  *   * * ** **
```

FIGURE 1F

```
CARNATION   AGCTTTATAAGTGTTGCTGAGGTTATAAA------GGACATGCCTCATGT
CARNATION   AGCTTTATAAGTGTTGCTGAGGTTATAAA------GGACATGCCTCATGT
PETUNIA     CAATTTGTAAGCATTGCTGAAATTCTCAACGAT---GAAAAAAGTAACTT
TOMATO      CAATTCGTCAGTATAGCTGAAATCCTCGATGAACAGGAAATGACTTACTG
ORCHID      AACTTATTCAGCATTTCAGATCTCATCACTGATGC---CATCTCTGA---
ARABIDOPSIS GATTTCGTGAGCGTTGCTGAGGTGGTCAATGATGTGGACATCTCCGAAGT
ZUCCHINI    ACCTTCACCAGCATCGCTGAGATTGTTGAACAAATGGAG------CATTG
                **   **  *  * **  *  *                      *

CARNATION   AAATCAAGACCTTGTTCATATTTTATATAGTTTGTCCAAGGACATGGGCA
CARNATION   AAATCAAGACCTTGTTCATATTTTATATAGTTTGTCCAAGGACATGGGCA
PETUNIA     CAACAAAGATTTGGTCCATATTGTCTATAGTCTCTCAAAAGACATGGGAT
TOMATO      CAACAAAGATTTAGTTCACATCGTCTACAGTCTTTCAAAAGACATGGGGT
ORCHID      --ACAA-------GTTCATATTGTTTATAGCTTATCGAAAGATTTGGGCC
ARABIDOPSIS CAACGTTGACTTGATTCACATTGTCTATAGTCTTTCTAAAGATATGGGAC
ZUCCHINI    CAAGAAGGAGCTCATCCATATTCTTTATAGCTTGTCCAAAGACATGGGCC
                *     * ** **  * ** **  * ** ** **  ****

CARNATION   TGCCGGGCTTTAGGGTTGGGATCATTTACTCTTATAATGACCGTGTCGTC
CARNATION   TGCCCGGCTTTAGGGTTGGGATCATTTACTCTTATAATGACCGTGTCGTC
PETUNIA     TACCAGGATTTCGAATTGGAATCGTATATTCTTACAACGATGCCGTTGTA
TOMATO      TACCAGGATTTAGAGTCGGAATCATATATTCTTTTAACGACGATGTCGTT
ORCHID      TTCCTGGTTTCAGAGTTGGAGCTCTGTATTCCTATAACGACAGAGTTGTT
ARABIDOPSIS TTCCTGGTTTTAGAGTCGGGATAGTCTATTCTTTCAATGACTCGGTCGTG
ZUCCHINI    TCCCTGGTTTTCGAGTTGGAATTATTTATTCTTACAACGATGTCGTCGTC
            * ** ** **  *  * **     * ** ** *  *  ** **    ** **
```

FIGURE 1G

```
CARNATION   TCAACTGCTCGTCGAATGTCGAGTTTTGGACTTGTTTCTTCTCAAACTCA
CARNATION   TCAACTGCTCGTCGAATGTCGAGTTTTGGACTTGTTTCTTCTCAAACTCA
PETUNIA     AATTGTGCACGAAAAATGTCAAGTTTCGGTTTAGTTTCCACTCAGACACA
TOMATO      AATTGTGCTAGAAAAATGTCGAGTTTCGGTTTAGTATCTACACAAACGCA
ORCHID      AAACAGCGAGAAGAATGTCAAGTTTCAGTCTTGTTTCTTCTCAGACTCA
ARABIDOPSIS TCTTGCGCAAGAAAAATGTCAAGTTTCGGACTTGTTTCGTCTCAGACACA
ZUCCHINI    CGCCGTGCTCGGCAGATGTCGAGCTTCGGCCTCGTCTCGTCCCAGACTCA
                **  *    ***** ** **  *  * ** **  * ** ** **

CARNATION   GTTTATGATCGCGGCATTGCTCTCAGATGATGATTTTGTTAGACGATTCT
CARNATION   GTTTATGATCGCGGCATTGCTCTCAGATGATGATTTTGTTAGACGATTCT
PETUNIA     ACACTTGCTAGCAAAAATGTTATCCGACGAAGAATTTGTCGCAAATTTTC
TOMATO      ATATTTTTTAGCGGCAATGCCATCGGACGAAAAATTCGTCGATAATTTTC
ORCHID      AAAGTTGCTGTCTTTTATGCTGTCAGATGAGGAGTTTACAGTGAGATATA
ARABIDOPSIS ACTCATGCTTGCTTCGATGTTGTCCGATGATCAGTTTGTGGATAATTTTC
ZUCCHINI    ACATTTGCTCGCCGCCATGCTTTCCGACGAGGACTTTGTCGACAAATTTC
                *  *  *     **   ** **  * **                *

CARNATION   TGGTTGAGAGTAGAGACAGACTCTTTCGAAGGCACCAGCATTTCACAAGC
CARNATION   TGGTTGAGAGTAGAGACAGACTCTTTCGAAGGCACCAGCATTTCACAAGC
PETUNIA     TTTGTGAAAGCTCAATGAGGTTAGGTAAAAGACATAAACATTTTACTAAT
TOMATO      TAAGAGAAAGCGCGATGAGGTTAGGTAAAAGGCACAAACATTTTACTAAT
ORCHID      TAGAGAAGAATAGAGAGAGACTGAGAGAGAGATATGAATTAGTTGTTAAT
ARABIDOPSIS TAATGGAAAGCTCGAGAAGGTTGGGGATAAGGCATAAAGTTTTACCACG
ZUCCHINI    TTGCCGAGAACTCGAAGCGTGTGGGCGAGAGGCATGCAAGGTTCACAAAA
            *     * *         * *     **  *        *     *
```

FIGURE 1H

```
CARNATION   GAGCTGGCTAAGATAGGAATAGGATGCCTCCAAGGAAACGCGGCATTGTT
CARNATION   GAGCTGGCTAAGATAGGAATAGGATGCCTCCAAGGAAACGCGGCATTGTT
PETUNIA     GGACTAGAACAAGTTGGTATTAAATGCTTGAAAAGCAATTCAGGACTTTT
TOMATO      GGACTTGAAGTAGTGGGAATTAAATGCTTGAAAAATAATGCGGGGCTTTT
ORCHID      GGGTTGAAGGAAGCAGGGATTGAGTGCTTGAAAGGAGAGGCAGGGCTGTT
ARABIDOPSIS GGGATCAAGAAAGCAGATATTGCTTGTTTGACAAGCAACGCTGGTTTATT
ZUCCHINI    GAATTGGATAAAATGGGGATCACTTGCTTGAACAGCAATGCTGGAGTTTT
            *   *      *  **   **  *     *  * *  *   * **

CARNATION   TGTTTGGATGGATTTGAGGCATCTATTAGACGAAGCAA---CGGTTGAAA
CARNATION   TGTTTGGATGGATTTGAGGCATCTATTAGACGAAGCAA---CGGTTGAGG
PETUNIA     CTGCTGGATGGATTTGCGGCACCTTTTGGAAAATTCCA---CGTTGGATT
TOMATO      TTGTTGGATGGATTTGCGTCCACTTTTAAGGGAATCGA---CTTTCGATA
ORCHID      CTGTTGGGTGAATATGGAGAAGTTGATGGAGGAGGAGA---CGAAGGAAG
ARABIDOPSIS TGCGTGGATGGATTTGAGACATCTACTGAGAGATCGTAACTCGTTTGAAT
ZUCCHINI    TGTGTGGATGGATCTACGGAGGCTATTAAAAGA-CCAAACC--TTCAAAG
               *** ** ** *     *  *    *        *

CARNATION   GAGAGTTAAAGTTATGGAGAGTGATCATCAATGAAGTGAAAATCAATGTG
CARNATION   GAGAGTTAAAGTTATGGAGAGTGATCATCAATGAAGTGAAAATCAATGTG
PETUNIA     CTGAAATGTCATTATGGAGAGTGATTATAAACGATGTGAGACTTAACGTT
TOMATO      GCGAAATGTCGTTATGGAGAGTTATTATAAACGATGTTAAGCTTAACGTC
ORCHID      GAGAAGCAGAGCTCTGGAAGGTGATAATTGATGATTTAAAGCTTAATATA
ARABIDOPSIS CTGAGATCGAGCTTTGGCATATAATCATCGATAGAGTTAAGCTCAATGTG
ZUCCHINI    CTGAAATGGAGCTTTGGCGTGTGATTATCAATGAAGTCAAGCTCAATGTT
               **      * ***   * ** **  *    * *   * **  *
```

FIGURE 11

```
CARNATION   TCACCGGGTTCGTCCTTCCTGTGCTCTGAGCCAGGGTGGTTTAGGGTTTG
CARNATION   TCACCGGGTTCGTCCTTCCTGTGCTCTGAGCCAGGGTGGTTTAGGGTTTG
PETUNIA     TCGCCTGGATCTTCATTTGATTGTCAAGAGCCAGGATGGTTTCGTGTTTG
TOMATO      TCGCTTGGATCTTCGTTTGAATGTCAAGAGCCAGGGTGGTTCCGAGTTTG
ORCHID      TCGCCAGGTTCTTCATGTTGTTGCTGAACCAGGTTGGTTCAGACTTTG
ARABIDOPSIS TCTCCTGGCTCTTCCTTCCGTTGCACGGAACCTGGATGGTTTAGGATTTG
ZUCCHINI    TCTCCTGGCTCATCCTTTCATGTCACTGAGCCAGGTTGGTTTCGAGTTTG
            ** *  ** ** ** *        ** ** ** *****  *  ****

CARNATION   CTTTGCCAACATGGACAATGCGACCTTAGACGTTGCACTCAATCGAATTA
CARNATION   CTTTGCCAACATGGACAATGCGACCTTAGACGTTGCACTCAATCGAATTA
PETUNIA     TTTCGCTAATATGGATGATGAAACAGTGGAAGTTGCACTAGCGAGAATAA
TOMATO      TTTTGCAAATATGGATGATGGAACGGTTGATATTGCGCTCGCGAGGATTC
ORCHID      CTTTGCTAATATGAGCAGAGAGACGTTGGAGGTGGCGCTGAAGAGATTGA
ARABIDOPSIS CTTTGCCAACATGGACGATGATACTCTCCATGTGGCGCTTGGACGGATCC
ZUCCHINI    TTTCGCAAACATGGACGACAACACCGTTGACGTTGCTCTCAATAGAATCC
              ** ** ** ***        **  *  *  * ** **  *  *

CARNATION   GGTCTTTTGTAACCCGTGGAAGGGTGGACAATTCAACAATGACACAACA
CARNATION   GGTCTTTTGTAACCCGTGGAAGGGTGGACAATTCAACAATGACACAACA
PETUNIA     GAAGGTTTGTG----------GGTG--------------------TTG
TOMATO      GGAGGTTCGTA----------GGTG--------------------TTG
ORCHID      AGGATTTTG---CTCAGAAGAAGGT-----------------------CG
ARABIDOPSIS AAGATTTCGT----------G-----------------------TC-
ZUCCHINI    ATAGCTTTGTC----------GAAA--------------------ACA
                ** *
```

FIGURE 1J

```
CARNATION   TCAGCAAGAGCAGCAGCAACAACAACAACAACAACAACAACAACAACAAC
CARNATION   TCAGCAAGAGCAGCAACAACAACAACAACAACAACAACAACAACAACAAC
PETUNIA     GTGTTAAGGAAAATGGAGAGGAGGA-GACTAAGCCAGTAAAGA-ACAAGGAGC
TOMATO      ------AGAAAAGTGGAGATAA-ATCGAGTTCGATGGAAA-AGAAGCAAC
ORCHID      CTGCTAAGAAGAA-GAAGATG-----------------------------
ARABIDOPSIS ----TAAGAACA---------AG-AACAAGATCG-TCGAGA-A-----AGC
ZUCCHINI    TCGACAAGAAGGAAGACAATAC-CGTTGCAATGCCATCGA-AAACGAGGC
                  **

CARNATION   AACAACAACAACAACAACGACAATTAAGAAGAAACGAGGGCAAATGGAGC
CARNATION   AACAACAACAACAACAACGACAATTAAGAAGAAACGAGGGCAAATGGAGC
PETUNIA     AATGG-AA-AAAG--AGTAAT--TTACGA---------------------
TOMATO      AATGG-AA-GAAG--AATAAT--TTGAGA---------------------
ORCHID      ----------------AACAATGTTTA-----------------------C
ARABIDOPSIS ATCTG-AA-AATG--A-----------------------------------
ZUCCHINI    ATCGA-GATAATA--AGTTACGATTGAGC---------------------
                           *

CARNATION   TTCGACTTAGCTTCAACAATCGAAGATTCGAAGACGGTTTAATGTCACCT
CARNATION   TTCGACTTAGCTTCAACAATCGAAGATTCGAAGACGGTTTAATGTCACCT
PETUNIA     GTTAGTTTCTCTCGAAAAGAATGTACGA--TGAAAGTG-TTT--TGTCACCA
TOMATO      CTTAGTTTTTCGAAAAGAATGTATGA--TGAAAGTG-TTT--TGTCACCA
ORCHID      TTCTGTT-----------------GATT--AATTAGACTTAGT--------
ARABIDOPSIS -TCAGGT----------AATCCAGAA--CAAGAGTG-CT-----------
ZUCCHINI    TTCTCCTTCTCAGGGAGAAGATACGA--CGAGGGCAACGT--TCTTAACT
              *   *                 *     *
```

FIGURE 1K

```
CARNATION   CATAGCATCCTATTATCTCCTCACTCTCCTATGCCTCAATCACCCCTTGT
CARNATION   CATAGCAGCCTATTATCTCCTCACTCTCCTATGCCTCAATCACCCCTTGT
PETUNIA     CTTT-CA-TCTCCTATA----------------CCTCACTCACCACTTGT
TOMATO      CTTT-CG-TCACCTATT----------------CCTCCCTCACCATTAGT
ORCHID      ----------------------------TGTACTTAAGTTGT--TTAAT
ARABIDOPSIS ----------------------------------------------
ZUCCHINI    CACCGCA-CACGATGTC----------------GCCTCACTCGCCGTTAGT

CARNATION   TAAAGCAAGAACATAAGTCTAAAATCATGAGTTATTAATAAATTTAT
CARNATION   TAAAGCAAGAACATAAGTCTAAAATCATGAGTTATTAATAAATTTAT
PETUNIA     TCGAGCAAGA-----ACTTGAAGAGGAAGAATT----ACC-GTA-GTTTT
TOMATO      TCGT-TAAGAC--TTAATTAAAAGGGAAGAATT--TAATTTATG-TTTTT
ORCHID      TAG----------TTAGATGAAAA-AGTAAGTTGT----------------
ARABIDOPSIS ------AAAA-----AGCTGAAATGGACGCA-----GACC---A-ATCTT
ZUCCHINI    AATAGCAAAAA-ATTAATTAAAAACATTTTTTCA--AAATATTCATACCAT
                          *     **

CARNATION   CGAACCAGTGTGACGCCATTGAAACGGTGCGACGGGAGTTGAAACGGTGT
CARNATION   CGAACCAGTGTGACGCCATTGAAACGGTGCGACGGGA-TTGAAACGGTGT
PETUNIA     CAATTTTATTATTGTTA----TTGAAATAAGGAAATGATA-TAGAAA------
TOMATO      TTATATTTTTGAAAAAAATTTGTAAGAATAAGATTATATAGGAA--------
ORCHID      --ACTTGGT-----------------------------TGGACAC--TTT
ARABIDOPSIS CGACT----------AAGTTTCCGACGAC---TTTAC---------------
ZUCCHINI    TCATATAGTTTTTTTTTTTTTTTTTGGGTCAATGTTGACTA-----
             *
```

FIGURE 1L

```
CARNATION   GAAAGACCACATTCAGATGAAGCATTATATCTTCTCTACAAACATTGAA
CARNATION   GAAAGACCACATTCAGATGAAGCATTATATCTTCTCAACAAACATTGAA
PETUNIA     -AAGAAA-A-GAGAATGTCGTAGGATAATTCTTTTCAG-AAAGAATTG--
TOMATO      -AAGAAA-ATAAGTATGTAGGATGAGGAGTATTTTCAG-AAATAGTTG--
ORCHID      GAGAAATTA----------ATGCTA----TTTTCATTGGTACGTACAA
ARABIDOPSIS ---GAGG-ATG-GTCTCTCG----------TCTCCAG-GGATAAT-G--
ZUCCHINI    -AAGTTACGTATATTTTTTCCACAGTGGATATGATGTA-AACTTCATA--
                                    *

CARNATION   CTTAATATAATTCAATATAACTTCTCTGTAATTTCATGTATACAAACACT
CARNATION   CTTAATATAATTCAATATAACTTCTCTGTAATTTCATGTATACAAACACT
PETUNIA     -TT---GTACTTATAGT--ATT---------A--TTA--ATAATTAG-----
TOMATO      -TTAGCGTATGTATTGACAACT---------GGTCTA-TGTACTTAGACAT-
ORCHID      GTTGATATG----------TGAGTTTTTAGTACTGT-ATATATCT-------
ARABIDOPSIS -TCACCACACTCACC----------TCT-----------------------
ZUCCHINI    -TTTTTTGGTGGGATGGTGATA---------GATGTAATGTATTTGGTTTTT
                                    *

CARNATION   ATAAATATGTAGTCATGTGTAAGATCATTGATATAGAAAAATATAAATGA
CARNATION   ATAAATATGTAGTCATGTGTAAGATCATTGATATAGAAAAATATAAATGA
PETUNIA     ----------TAAT-GTATTTTGACTTGTTTAAT-TTA-----------
TOMATO      ----------CATAAT-TTGTCTTAGCTAATTAAT-GAATG---CAAAAGTGA
ORCHID      ----------TAATTGCATTTC----------ATGAAATAAATGTTA
ARABIDOPSIS ----------TCTC----------CGAGCATGA
ZUCCHINI    CC-----CTTAGG-GAACTCATACTTATTTAT-TAATGAAATGATTGTGA
```

FIGURE 1M

```
CARNATION   TTTTCTGATTTTAAAAAAAAA
CARNATION   TTTTCTGATTTTA---------
PETUNIA     ----------------------
TOMATO      AGT-----T-------------
ORCHID      ATT----ATT------------
ARABIDOPSIS ----------------------
ZUCCHINI    TTT----AT-------------
```

FIGURE 1N

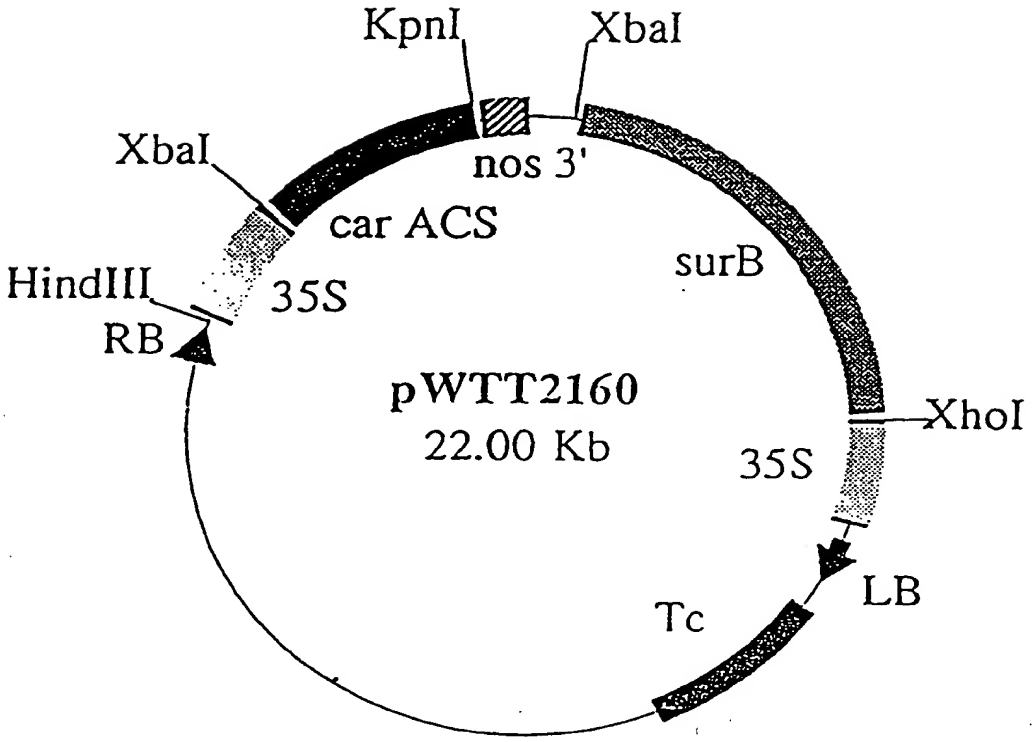
KpnI
XbaI
XbaI
nos 3'
car ACS
surB
HindIII
35S
RB
pWTT2160
22.00 Kb
XhoI
35S
LB
Tc

FIGURE 2

17/40

| |
|---|
| 18/40 |
| 19/40 |
| 20/40 |
| 21/40 |
| 22/40 |
| 23/40 |
| 24/40 |
| 25/40 |
| 26/40 |
| 27/40 |
| 28/40 |

```
CARNATION  ----------------------------------------AAAACA
CARNATION  ----------------------------------------C-
ARABIDOPSIS CGTT----------GCTGTC----------GAAG---
TOMATO     TTTTTTTTTTACACTCCAAATTATTATTTGTATAATTTCACTAGAAGGCA
ORCHID     ----------AAC---AAACAGCT----------TAAGAG---
APPLE      TTT---------AG---TAATCT----------TA---AA----------ACA
PETUNIA    ----------GTTATTCACA----------CACTA----------
SUNFLOWER  C----------AACTGCAAACA----------CACACAT----------ACA
GERANIUM   CTT---------GAGTCTTGAGTG----------TGTGTTA----------GCA

CARNATION  ----------AATACAAATACAAATAC----------AAATACATTGAATT
CARNATION  ----------TACAAATAC----------AAATACATTGAATT
ARABIDOPSIS ----------TTAGG----------CCAAGAAACCCATTTA---
TOMATO     CCTTATTTATAGATACAAATATTAGGACTCTTTTTTGATACATATTCATCT
ORCHID     -CTT-TCT----------CTCTCTCAA----------TAAGCT
APPLE      ----------AATTTCAAAT-TTATCA----------AGTGCA----------
PETUNIA    ---------TATA----------CTCTAAAAACACATTC----
SUNFLOWER  ----------A-ACACACACAT-AGCA----------AAAGAAT-CA----------
GERANIUM   ----------AGAAACAAACATTAGTG----------TGAAAACACA----------

CARNATION  TGTTA----------ATTAACGAAC----------AT--GGCAAACATTGTCAA
CARNATION  TGTTA----------ATTAACGAAC----------AT--GGCAAACATTGTCAA
ARABIDOPSIS ----------AAAAAA
TOMATO     CTTCAATCTTTTGTATTCACATATTCTATTTATTCAATACACTTAGGAAA
ORCHID     TTC----CTCTTGAAACAGTGAGATCTAGTGA---GAGAAACA----------
APPLE      ----AAT----------ACATA----------AACACA----------A-
PETUNIA    -TTCA----------TTCTG----------
SUNFLOWER  ----ATT----------TCAAG----------AACAAA----------A-
GERANIUM   ----AGA----------GAAGG----------AGAAAA----------AA
```

FIGURE 3A

```
CARNATION     CTTCCCTATC-----ATTGACATGGAGAAGCTC--AATAATTA-------
CARNATION     CTTCCCTATC-----ATTGACATGGAGAAGCTC--AATAATTA-------
ARABIDOPSIS   A-------------AGAGAGAGATGGAGAGTTTCCCGATCATCAATCTCGA
TOMATO        ACACTTTACCAAGAAATTAAGATGGAGAACTTCC-AATTATTAACTTGAA
ORCHID        ----------GAGAGATGGAGAGCGGAAGCTTCCCTGTAATTAACATGGA
APPLE         ----------TCC-AAAGAGAAATGGCGACTTTCCCAGTTGTTGACTTGAG
PETUNIA       ----------CAAGAAAG-AAAATGGAGAACTTCCCAATTATCAGCTTGGA
SUNFLOWER     ----------GAGATGGCAAACTTCCCAGTTATCAACATGGA
GERANIUM      ATACCTTGCTTT-TATTGGAGATGGAGAGCTTTCCAGTGATCAACATGGA
                         * * * * * * * *

CARNATION     ------TAATGGTGTTGAGAGGAGTCTTGTTTTGGACCAAATTAAGGATG
CARNATION     ------TAATGGTGTTGAGAGGAGTCTTGTTTTGGACCAAATTAAGGATG
ARABIDOPSIS   GAAGCTTAATGGAGAAGAGAGCAATCACTATGGAGAAGATCAAAGACG
TOMATO        -AAGCTCAATGGAGATGAGAGAGCCAACACCATGGAAATGATCAAAGATG
ORCHID        GCTTCTCCAGGGTTCCCAGCGCCCGGCCGCCATGGCTCTTCTCCGAGACG
APPLE         CCTTGTCAATGGTGAAGAGAGAGCAGCAACCTTGGAGAAGATCAATGATG
PETUNIA       CAAAGTGAATGGTGTTGAAAGAGCTGCCACTATGGAAATGATTAAGGATG
SUNFLOWER     GAACCTGAATGGTTCTGAGAGAGGTGTTACCATGGAGAAGATCAATGATG
GERANIUM      GAAGTTGAATGGTGAGGAGAGAGCAGCAACCATGGAGAAGATCAAGGATG
                    * **    *  *      ***     *     ** *

CARNATION     CTTGTCACAACTGGGGATTCTTCCAGGTGGTGAACCATAGTTTGTCACAT
CARNATION     CTTGTCACAACTGGGGATTCTTCCAGGTGGTGAACCATAGTTTGTCACAT
ARABIDOPSIS   CTTGTGAAAACTGGGGCTTCTTTGAGTGTGTGAACCATGGGATTTCACTC
TOMATO        CTTGTGAGAATTGGGGCTTCTTTGAGTTGGTGAACCATGGAATTCCACAT
ORCHID        CCTGTGAGAACTGGGGTTTGTACGAGTTACTGAACCACGGAATCTCCCAC
APPLE         CTTGTGAGAACTGGGGTTTCTTTGAGCTGGTGAACCATGGGATGTCTACT
PETUNIA       CATGTGAAAACTGGGGCTTCTTTGAGTTGGTGAACCATGGAATCCCACGT
SUNFLOWER     CATGTGAAAACTGGGGATTCTTTGAGTTGGTGAACCATGGGATTCCTCAT
GERANIUM      CTTGTGAAAACTGGGGTTTTTTTGAGCTGTTGAACCATGGGATACCCTAT
              * *** * ** ***** ** * *  **    *******  *  * *
```

FIGURE 3B

```
CARNATION     GAACTGATGGACAAAGTGGAGAGGATGACAAAAGAGGCATTACAAGAAATT
CARNATION     GAACTGATGGACAAAGTGGAGAGGATGACAAAAGAGGCATTACAAGAAATT
ARABIDOPSIS   GAGCTTTTGGACAAAGTGGAGAAGATGACCAAGGAACATTACAAGAAGTG
TOMATO        GAAGTAATGGACACAGTAGAGAAAAATGACAAAGGGACATTACAAGAAGTG
ORCHID        GAGCTAATGAACCGGGTGGAGACGGTAAACAAAGAACATTACCGGCGGTT
APPLE         GAGCTTTTGGACACTGTGGAGAAGATGACCAAGGATCACTACAAGAAGAC
PETUNIA       GAAGTAATGGACACTGTGGAGAAGATGACAAAGGGTCATTACAAGAAGTG
SUNFLOWER     GATTTACTTGACAAAGTCGAAAAGATGACAAAGGATCATTACAAGAAGTG
GERANIUM      GAGCTGCTTGACACAGTGGAGAAGATGACAAAGGAGCATTACAGGAAGTG
              **  *  *  **  ** ** *  * *  ** *  ** *** *

CARNATION     CAGGGAGCAAAAGTTCAAAGACATGGTTCAGACCAAAGGTTTAGTGTCTG
CARNATION     CAGGGAGCAAAAGTTCAAAGACATGGTTCAGACCAAAGGTTTAGTGTCTG
ARABIDOPSIS   CATGGAAGAGATTCAAGGAATCGATTAAGAACAGAGGTCTTGACTCTC
TOMATO        CATGGAACAGAGGTTTAAGGAACTAGTGGCAAGTAAGGGACTTGAGGCTG
ORCHID        CCGGGAACAGCGCTTCAAAGAATTCGCGTC---CAAAACCCTAGATACCG
APPLE         CATGGAGCAAAGGTTTAAGGAAATGGTGGCAGCCAAAGGCCTCGACGATG
PETUNIA       CATGGAACAAAGGTTCAAGGAATTGGTTGCCAGTAAAGCTCTTGAAGGTG
SUNFLOWER     TATGGAGCAGAGGTTTAAGGAAATGGTGGCAGCCAAAGCTTTAGAAGGTG
GERANIUM      TATGGAGCAGAGGTTTAAGGAAATGGTGGCAAGCAAGGGACTTGAAGGAG
                 ***  *   ** ** **          *       * *

CARNATION     CTGAG---TCTCAAGTCAATGACATTGATTGGGAGAGCACCTTCTACCTT
CARNATION     CTGAG---TCTCAAGTCAATGACATTGATTGGGAGAGCACCTTCTACCTT
ARABIDOPSIS   TTCGC---TCTGAAGTCAACGACGTTGACTGGGAATCCACTTTCTACCTC
TOMATO        TTCAA---GCTGAGGTTACTGATTTAGATTGGGAAAGCACTTTCTTCTTG
ORCHID        TGGAGAACGTCGAGCCGGAAAATCTGGACTGGGAGAGCACCTTCTTCCTC
APPLE         TCCAG---TCCGAAATCCACGACTTGGACTGGGAAAGCACCTTCTTCTTG
PETUNIA       TTCAA---GCTGAGGTTACTGATATGGATTGGGAAAGCACCTTTTTCTTG
SUNFLOWER     TGAAA---GCCGAAGTTACCGATATGGATTGGGAGAGCACTTTCTTCTTG
GERANIUM      TGGAG---GTAGAGGTTGAGGACTTGGATTGGGAGAGCACTTTTTCTTG
                       *   *  * ** *****   *** ** * * *
```

FIGURE 3C

```
CARNATION       CGTCATCGTCCCACCTCCAACATCTCCGAGGTCCCTGATCTCGACGACCA
CARNATION       CGTCATCGTCCCACCTCCAACATCTCCGAGGTCCCTGATCTCGACGACCA
ARABIDOPSIS     AAGCACCTTCCCGTCTCTAATATCTCCGATGTCCCTGATCTCGACGACGA
TOMATO          CGCCATCTTCCTACTTCTAATATCTCTCAAGTACCCGATCTTGACGAAGA
ORCHID          CGCCATCTCCCCACCTCCAACATCTCCCAAATCCCCGATCTGGATGACGA
APPLE           CGCCACCTTCCTTCCTCAAACATTTCCGAAATCCCTGATCTCGAGGAAGA
PETUNIA         AAACATCTCCCCATTTCTAACATTTCTGAAGTCCCTGATCTTGATGAAGA
SUNFLOWER       CGCCATCGCCCTACCTCCAACATATCCGAGATCCCTGATCTTGTAGATGA
GERANIUM        AAGCATCTCCCAGAATCAAACATCTCTCAAGTCCCTGATCTTCAAGACGA
                   ** *  **    ** ** ** **  *  * ** *****    **  *

CARNATION       ATACAGGAAGTTGATGAAGGAGTTTGCAGCCCAGATTGAGAGGTTATCCG
CARNATION       ATACAGGAAGTTGATGAAGGAGTTTGCAGCCCAGATTGAGAGGTTATCCG
ARABIDOPSIS     TTACAGAACGTTAATGAAAGACTTCGCCGGAAAGATAGAGAAGTTGTCGG
TOMATO          ATACAGAGAGGTGATGAGAGATTTTGCTAAAAGATTGGAGAAATTGGCTG
ORCHID          TTGCCGGTCAACCATGAAGGAATTCGCGCTGGAGCTAGAGAACCTCGCGG
APPLE           GTACAGGAAGACCATGAAGGAATTTGCAGTGGAACTGGAGAAGCTAGCTG
PETUNIA         ATACAGGGAAGTTATGAGAGATTTTGCTAAAAGGTTAGAGAAGCTGGCAG
SUNFLOWER       ATACAGGGAGTTGATGAAGGATTTTGCTGGTAAATTAGAGAAGCTAGCAG
GERANIUM        GTACAGGAAGGTGATGAAGGAATTTGCAGCAAAACTAGAGAAACTAGCCG
                  * * *       ****  ** ** **        * ****   *  * *

CARNATION       AGCAACTGTTGGACTTGTTATGTGAGAACCTTGGCCTTGAGAAAGGCTAC
CARNATION       AGCAACTGTTGGACTTGTTATGTGAGAACCTTGGCCTTGAGAAAGCGTAC
ARABIDOPSIS     AGGAGCTACTGGATCTGCTGTGCGAGAATCTCGGTTTAGAGAAGGGTTAT
TOMATO          AGGAGTTACTTGACTTACTCTGTGAAAATCTTGGACTTGAAAAAGGTTAC
ORCHID          AGAGACTGCTGGATCTGTTGTGCGAAGATTTGGGGCTGGAGAAAGGGTAT
APPLE           AGAAGCTTTTGGACTTGCTGTGTGAGAATCTTGGACTCGAGAAGGGTTAT
PETUNIA         AGGAGCTTTTGGACTTGCTGTGTGAAAATCTTGGCCTGGAAAAAGGTTAC
SUNFLOWER       AGGAGCTTTTGGATTTATTATGTGAGAATCTTGGGTTGGAGAAAGGTTAC
GERANIUM        AGGAGCTACTAGACCTGTTGAGCGAGAATCTTGGGCTAGAGAAAGGTTAC
                **    *  * **  *  *  * **  *  * **  * ** ** *  **
```

FIGURE 3D

```
CARNATION   CTTAAGAATGCCTTCTATGG---TGCCAATGGC---CCCACTTTTGGTAC
CARNATION   CTTAAGAATGCCTTCTATGG---TGCCAATGGC---CCCACTTTTGGTAC
ARABIDOPSIS TTAAAAAAAGGTGTTTTACGG---GTCGAAAAGA---CCGACTTTTGGAAC
TOMATO      TTGAAAAAATGCCTTTTATGG---ATCAAAAGGT---CCCAACTTTGGTAC
ORCHID      TTGAAAAAGGTTTTCTGCGGGGGATCGGATGGTTTGCCGACGTTCGGGAC
APPLE       CTGAAGAAGGTTTTCTATGG---ATCCAAGGGT---CCGAATTTTGGGAC
PETUNIA     CTTAAAAAATGCCTTTTATGG---ATCGAAAGGT---CCAAACTTTGGGAC
SUNFLOWER   TTAAAGAAAGCCTTTTATGG---TTCAAAGGGT---CCAAACTTTGGAAC
GERANIUM    CTGAAAAAAGCTTTCTATGG---CTCAAAGGGT---CCAACCTTTGGCAC
              * ** ** *  ** *  **    *  *  *     ** *  ** ** **

CARNATION   CAAGGTCAGCAACTACCCGCCTTGCCCCCAAACCCGACCTTATCAAAGGAC
CARNATION   CAAGGTCAGCAACTACCCGCCTTGCCCCCAAACCCGACCTTATCAAAGGAC
ARABIDOPSIS CAAAGTCAGCAATTATCCACCTTGTCCTAATCCGGACCTAGTCAAGGGTC
TOMATO      TAAAGTTAGCAACTATCCACCATGTCCTAAGCCCGATTTGATCAAGGGAC
ORCHID      GAAGGTGAGTAATTATCCGCCATGTCCGAAGCCGGAGCTGATAAAGGGAT
APPLE       CAAGGTCAGCAACTACCCTCCATGCCCCCAAGCCAGACCTGATCAAGGGAC
PETUNIA     TAAAGTGAGCAACTTACCACCATGCCCCAAAACCAGATTTAATCAAAGGAC
SUNFLOWER   CAAGGTTAGCAACTACCCACCATGCCCCAACACCGGATTTGATCAAGGGTC
GERANIUM    CAAGGTCAGCAACTACCCTCCCTGCCCCCAAGCCAGACTTAATCAAGGGAC
              ** ** ** ** *  ** ** ** ** *  ** **  *  *  * ** **

CARNATION   TTAGGGCCCCACACCGACGCTGGTGGCATCATTCTCTTGTTCCAGGACGAC
CARNATION   TTAGGGCCCCACACCGACGCTGGTGGCATCATTCTCTTGTTCCAGGACGAC
ARABIDOPSIS TCCGAGCCCCACACCGACGCCGGCGGCATCATCCTCCTCTTCCAAGACGAC
TOMATO      TCCGCGCTCATACAGACGCAGGAGGCATCATACTTCTGTTCCAAGATGAC
ORCHID      TGAGAGCTCATACGGATGCAGGGGGGGATCATTCTGTTGTTTCAGGATGAT
APPLE       TCCGGGCCCCACAGCGACGCCGGTGGCATCATCCTGCTTTTCCAGGATGAC
PETUNIA     TACGTGCCCCACACAGACGCTGGTGGAATAATCCTTCTGTTCCAAGATGTT
SUNFLOWER   TCCGAGCCCCATACCGATGCTGGTGGCATCATTTTGCTCTTTCAAGATGAT
GERANIUM    TCAGGGCACATACAGATGCCGGAGGCCTCATATTGCTCTTCCAAGACGAC
            *  *  ** ** *  ** ** ** **  *  **  *  *  ** ** ** *
```

FIGURE 3E

```
CARNATION   AAGGTCAGCGGCCTCCAGCTCCTCAAGGATGGTCATTGGGTTGATGTTCC
CARNATION   AAGGTCAGCGGCCTCCAGCTCCTCAAGGATGGTCATTGGGTTGATGTTCC
ARABIDOPSIS AAAGTCAGTGGACTTCAGCTTCTTAAAGACGGCGAGTGGGTCGATGTTCC
TOMATO      AAAGTGAGTGGCCTTCAACTCCTCAAAGACCGAGCAATGGATCGATGTTCC
ORCHID      AAGGTTAGCGGGCTTCAGTTGCTCAAGGACGGGGAATGGATCGATGTTCC
APPLE       AAGGTCAGCGGCCTCCAGCTTCTCAAGGATGGTGAATGGGTGGATGTCCC
PETUNIA     AAAGTAAGTGGCCTACAACTCCTCAAAGACGGCCAATGGATCGATGTTCC
SUNFLOWER   AAAGTTAGCGGTCTACAGCTTCTTAAGGACGGCGAATGGATCGATGTTCC
GERANIUM    AAGGTCAGTGGTCTCCAGCTCCTGAAAGACGGGAAGTGGGTCGATGTTCC
            ** ** ** ** ** **  * ** ** ** *   * *** * ***** **

CARNATION   TCCCATGAAACACTCCATTGTTGTTAACTTGGGGGACCAACTTGAGGTTA
CARNATION   TCCCATGAAACACTCCATTGTTGTTAACTTGGGGGACCAACTTGAGGTTA
ARABIDOPSIS TCCGGTTAAGCATTCAATCGTCGTTAATCTCGGCGATCAACTTGAGGTGA
TOMATO      TCCCATGCGCCACTCTATTGTGGTTAACCTTGGTGACCAACTTGAGGTGA
ORCHID      TCCCGTGCGCCATTCCATTGTCGTCAATATTGGAGATCAGCTGGAGGTGA
APPLE       CCCAATGCACCACTCCATTGTCATAAACTTAGGTGACCAGATTGAGGTGA
PETUNIA     TCCCATGCGCCATTCCATTGTGGTTAACCTCGGTGACCAACTCGAGGTGA
SUNFLOWER   GCCCATGCGCCATTCCATTGTCATCAATCTTGGTGACCAAATTGAGGTGA
GERANIUM    TCCTATGCACCACTCCATCGTCATCAACCTCGGTGACCAACTTGAGGTGA
               **  *    ** ** ** **  * **  * ** ** **  * ***** *

CARNATION   TTACAAATGGCAAGTACAAGAGTGTGATGCACCGCGTGATAGCGCAGACA
CARNATION   TTACAAATGGCAAGTACAAGAGTGTGATGCACCGCGTGATAGCGCAGACA
ARABIDOPSIS TAACCAATGGGAAGTACAAGAGTGTGGAACATAGAGTGCTATCTCAGACA
TOMATO      TCACTAACGGGAAGTACAAGAGTGTGCTGCACAGAGTAATTGCACAAACA
ORCHID      TAACGAATGGAAAATACAAGAGTGTGTTGCATAGGGTGGTGGCCCAAACC
APPLE       TCACCAATGGGAAGTACAAAAGTGTGATGCACCGGGTGATAGCTCAGTCG
PETUNIA     TCACTAATGGAAAATACAAGAGTGTGATGCACAGAGTGATAGCCCAAAAA
SUNFLOWER   TCACAAATGGGAAATACAAGAGTGTGATGCACAGAGTTATTGCTCAAACA
GERANIUM    TTACCAATGGGAAATACAAGAGCATAGAGCACCGTGTGATAGCCCAATCA
            * ** ** ** ** ***** **  *    **  * ** *  *  * **
```

FIGURE 3F

```
CARNATION      GATGGTAA---CAGGATGTCGATAGCATCATTCTACAACCCGGGAAGTGA
CARNATION      GATGGTAA---CAGGATGTCGATAGCATCATTCTACAACCCGGGAAGTGA
ARABIDOPSIS    GACGGAGAAGGAAGAATGTCGATCGCATCATTCTATAATCCGGGAAGCGA
TOMATO         GACGGGA---CACGAATGTCATTAGCCTCATTTTACAATCCAGGAAGTGA
ORCHID         GATGGAAA---CCGCATGTCGATCGCGTCGTTCTACAACCCTGGCAGCGA
APPLE          GATGGGA---CCAGAATGTCGATAGCCTCGTTCTACAACCCAGGCAACGA
PETUNIA        GACGGTG---CTCGAATGTCATTAGCTTCCTTTTACAATCCAGGAAGTGA
SUNFLOWER      GATGGAA---CAAGAATGTCAATTGCCTCTTTTACAACCCAGGGAATGA
GERANIUM       GACGGTA---CTAGAATGTCCATTGCTTCCTTCTACAACCCGGGAAGTGA
               **  **       * *****  * **  ** **  ** **  * *  **

CARNATION      TGCCGTGATTTACCCGGCGCCAACATTGGTGGAAAAGAAG----------
CARNATION      TGCCGTGATTTACCCGGCGCCAACATTGGTGGAAAAGAAG----------
ARABIDOPSIS    CTCTGTTATTTTTCCGGTGCCGGAGCTGATCGGAAAAGAAG----------
TOMATO         TGCAGTAATATATCCAGCAAAAACTTTGGTTGAAAAAGAGG----------
ORCHID         CGCCGTGATCTTTCCGGCGCCGGCGCTGGTGGAGAAAGAGGCGGAGGAGA
APPLE          CTCATTCATCAGCCCAGCACCGGCAGTGCTTGAGAAGAA----------
PETUNIA        TGCAGTGATCTATCCAGCACCAGCTCTTGTTGAGAAAGAAG----------
SUNFLOWER      TGCTGTGATCTATCCAGCTCCAACATTGTTGGAGAAGGAGC----------
GERANIUM       TGCGGTCATCTATCCAGCACCAGCTCTGTTGGAGAAAGA----------
                  *  * **   ** *       *  * *  **  *

CARNATION      AG---GAGAAATGCA---GAGCATACCCAAAATTTGTGTTCGAGGATTAC
CARNATION      AG---GAGAAATGCA---GAGCATACCCAAAATTTGTGTTCGAGGATTAC
ARABIDOPSIS    CAGAGAAGGAGAAGAAGAGAACTATCCGAGATTTGTGTTTGAAGATTAC
TOMATO         ---CAGAGGAAAGTACACAAGTGTATCCAAAGTTTGTGTTTGATGATTAC
ORCHID         AGGAGGAGAAGAAGGAAATTTATCCAAAGTTTGTGTTCCAGGATTAC
APPLE          -AACTGAGGACG---CCCCAACTTATCCCAAGTTTGTGTTTGATGACTAC
PETUNIA        AAGCAGAGAAAAACACAAGTCTACCCAAAATTTGTATTTGATGATTAC
SUNFLOWER      CAACAGAGAAAG---AACAATCGTACCCGAAATTCGTGTTTGATGATTAC
GERANIUM       -AACAGAAGAGA---AGCAAGTGTACCCGAAATTCGTGTTCGAAGACTAC
                        *  *          ** ** *  ** **  * ** ***
```

FIGURE 3G

```
CARNATION   ATGAATCTCTACTTAAAGCTCAAGTTCCAAGAGAAGGAGCCCAGGTTTGA
CARNATION   ATGAATCTCTACTTAAAGCTCAAGTTCCAAGAGAAGGAGCCCAGGTTTGA
ARABIDOPSIS ATGAAACTCTACTCTGCTGTCAAGTTTCAGGCCAAGGAACCAAGGTTTGA
TOMATO      ATGAAGTTATATGCTGGACTCAAGTTTCAAGCCAAAGAGCCAAGATTTGA
ORCHID      ATGAATCTGTACATTCGGAAGAAATTTGAGGCGAAGGAGCCGAGATTTGA
APPLE       ATGAAGCTGTATTCTGGCCTGAAATTCCAAGCCAAGGAGCCAAGATTTGA
PETUNIA     ATGAAGTTATATGCTGGACTCAAGTTTCAAGCTAAGGAGCCAAGATTTGA
SUNFLOWER   ATGAAACTGTATGCAGGTTTAAAGTTTCAGGCAAAGGAGCCAAGGTTTGA
GERANIUM    ATGAAGCTCTACTCTGGCCTCAAGTTCCAAGCCAAAGAGCCCAGATTTGA
            ***** * **      ** **  * *  ** ** ** ** ** *****

CARNATION   AGCA-ATGAAGGCCATGGAAACCA----------CGGGTCCCATTCCAACT
CARNATION   AGCA-ATGAAGGCCATGGAAACCA----------CGGGTCCCATTCCAACT
ARABIDOPSIS AGC-CATGAAAGCTATGGAGACAACTGTGGCCAACAAT---GTTG-GACC
TOMATO      AGCA-ATGAAGGCAATGGAAAGTGAT----------CCAATTGCAAGT
ORCHID      GGCG-ATGAAGAGTATGGAGATTGTTATGAGCTCTCAGCCGATACCCACT
APPLE       AGCT-ATGAAGGCCAAGGAATCCACC----------CCTGTTGCAACT
PETUNIA     AGCA-ATGAAAGCCATGGAAACTGATGTCAAGATGGATCCAATTGCCACT
SUNFLOWER   AGCCCATGAAAGCAGTTGAAGCTAATGTTGGCTTGGGTCCAGTTGCAACA
GERANIUM    AGCC-ATGAAAGCTGTGGAGGCTAATGTTACTTTGGATCCAATTCGAACT
              **  *****      **                *       *

CARNATION   GCTTGAAATAATGATTTGATTTGATATAATGCAATGCTTCTCATCAACCA
CARNATION   GCTTGAAATAATGATTTGATTTGATATAATGCAATGCTTCTCATCAACCA
ARABIDOPSIS ATT-----GGCCACTGCGTGAATGATAT-GTAACTGGTTAATAAATATAT
TOMATO      GCTTA---GATCCCAATTCAATTAAAAAAATTGGTGTTTGAAA--AATAT
ORCHID      GCTTGATTGGTTCATTAATGGCTATTGTTTGTGAAGAATTTAGGGCATAG
APPLE       GCCTGAG----CTCTG-AGAGCCGTCCGAGAACAT--GGCACAAAAATA-
PETUNIA     GTCTAATAGATGGAAGTTGGATAAAAATGATTTGTTATTAAAAGTAGTAC
SUNFLOWER   GTTTA-AGTGACAT----------GTTTTAAATAATAGAATGTGATAATA-
GERANIUM    GCCTAGAAAGATATTATACAACAACCTTAGCAGATCAGAAAGAAGAAGA-
```

FIGURE 3H

```
CARNATION   A--TTTAAGTATTTCT--AATATACGCCACTCTCATCTCATCTATAT
CARNATION   AGGTTTAAGTATTTCT--AATATACGCCACTCTCATCTCATCTATAT
ARABIDOPSIS AT-ATATATA-TATAT--ATAGTCTTT-------ATATAATGTCTT----
TOMATO      A---TTTA-AATATA---GCAATCTATGTATACA-CATTATTTGCTCTTC
ORCHID      GGTTTAATCTATGTGTTTGTGGTTTGCTTGAGTCTAGTGGTTTGATTTAT
APPLE       ATTAAAAATTAGGGTAT--GGGTTTTATCGTT-------T-TGTGCCAAAT
PETUNIA     AAGACCTGTGACATAT--ATTATATGTTTCTTTAGTATAGTGTGATCAAT
SUNFLOWER   ATTATTATTA--TTAT--ACTTTTTTTCTTT---TATC-TTTTTTCAAT
GERANIUM    ACAAAGGGTAGACTGT--GTTGTCTGTTCTTAAGGTGGT-TGTGTTGTGT
                      *     *                  * *

CARNATION   TCATATTCATATTATTAGTGTTTGTTGAATAAGAGCTTCC-----TTTTA
CARNATION   TCATATTCATATTATTAGTGTTTGTTGAATAAGAGCTCCT-----TTTTA
ARABIDOPSIS ----------AGAAACTTGATTATTCACTATACGAA--T-------AATT-
TOMATO      TTA--TGTATGGTAGAATAAAGTTAGTATTTAAAAAAG---------AT--
ORCHID      GTGAATTGTTTGGGGGCTTATCATTTATATTTATATGTGTAGTTGTTA
APPLE       A-AAGTTGGGGGAGAAG----------AAAGT--T-------TG---
PETUNIA     CTA--TTTACAAGAGGGGTGTGTCCCTACTATATATG--T-------AGT--
SUNFLOWER   TT---TTTTTGAGAGGTCTCAGCCATCTATAGATACT--C-------TACAA
GERANIUM    CCAGGCTGCTAAAAGCTTTGTGATTTGTTTTTAAATT--T-------TATGA

CARNATION   AGT----------------------------------------------
CARNATION   AGTATGATTGTTAA--TGTAATGTTCCATGTCCTATGGATTGT-ATGGT-
ARABIDOPSIS ----TTGTTCATGT--TGTTGTATGTTTAAGTGGTGAATGT---GTTAT-
TOMATO      --TGTGATTTGCTG--CA-TATGTATCAAAAAGA--GTCCTAATATT-T-
ORCHID      TGTATGGATAGTTTGCTATTAAATTTGTTCCTTGGGGTTTGGGCGTAATT
APPLE       -----GGTTTATTT--TGTT---TTCTTTATTTTTTATTCCACTGT----
PETUNIA     --TGTC-CCTACTA--CA-TATGTAGTTAAGTGG----CCTAAAGTT-T-
SUNFLOWER   AGAATTGTGTGCT---TA-----CTTTTTACTTGTTATACCATAATGAC-
GERANIUM    CGCACGGCTTACTA--TAATGGGTTCTTTATCAGTTTGTTTATAGTCAT-
```

FIGURE 3I

```
CARNATION   ----------------------------------------------------
CARNATION   -CTACACTAAT-A-CGGAGTATT-C--ATTCA-AATTACAATAATTTATC
ARABIDOPSIS -ATATGGGAATT----------------AATGTTTTTCT-GT-TCGAAAAAAA
TOMATO      -GTAT-CT-ATA----------AAT-AAGGTGCCTTCTAGTG-AAATTATAC
ORCHID      GAAGTGTGAGCTGATGTAGTCTTTGTTGTTCATCCCTTGAATATTTATTG
APPLE       ----------TTG-TGTAG--TT-GAAATCTATC--TATATAAAATACAA
PETUNIA     -GTAT-CTTATAC-TAAACTGAT-AAAGCTTCTTTTTACA-CAAATATTA
SUNFLOWER   -AAAAGTGTGTTG-TACTCATTT-TCAATTAATTATTA-ATTGAGCTTTG
GERANIUM    -GGGTGCTAATTA-TTTGGTATT-ATAATATATAAGAGTATTTAGTCAAAA

CARNATION   ----------------------
CARNATION   CC-CTTAAAAAAAAAAAAAA
ARABIDOPSIS AA-A----------AAAAA
TOMATO      AA-ATAATAATTTGGAGTGT
ORCHID      GCCACTAAATTTATTATTTA
APPLE       AT-GTTTGCTCCACAAAAA
PETUNIA     AT-ATACCCGACAGCAACG-
SUNFLOWER   AA-TCATGTAGTGTAATATC
GERANIUM    AA-AAAAAAAAAAAAAAAA
```

FIGURE 3J

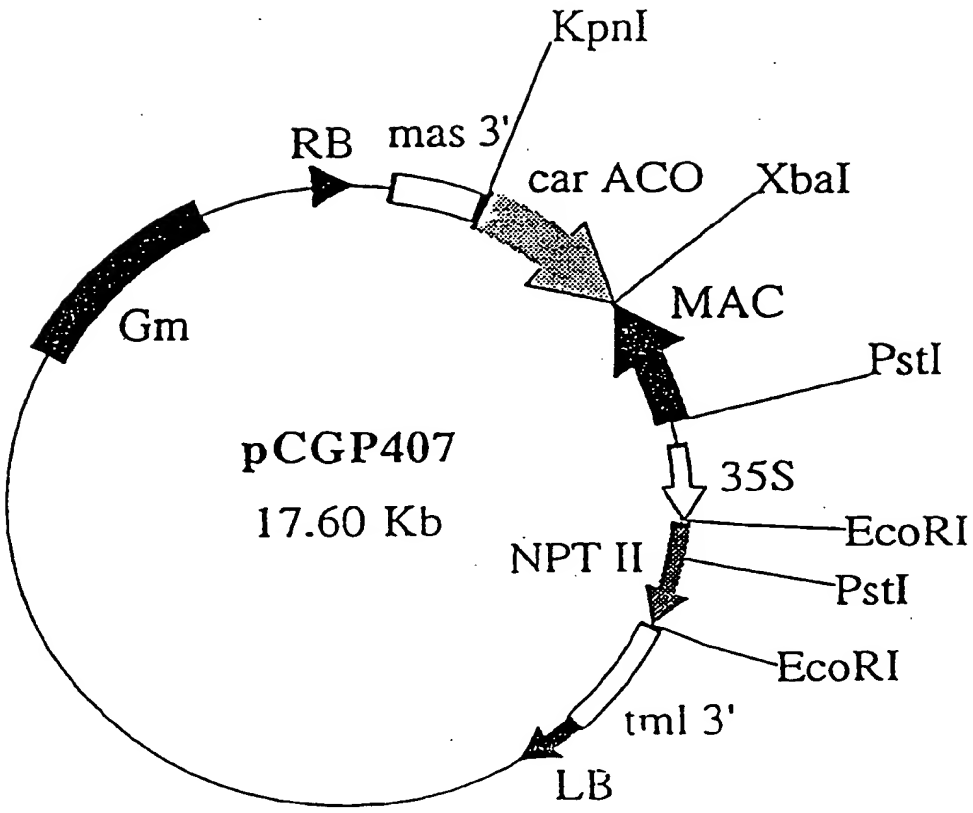
KpnI
RB
mas 3'
car ACO
XbaI
MAC
Gm
PstI
pCGP407
17.60 Kb
35S
EcoRI
NPT II
PstI
EcoRI
tml 3'
LB

FIGURE 4

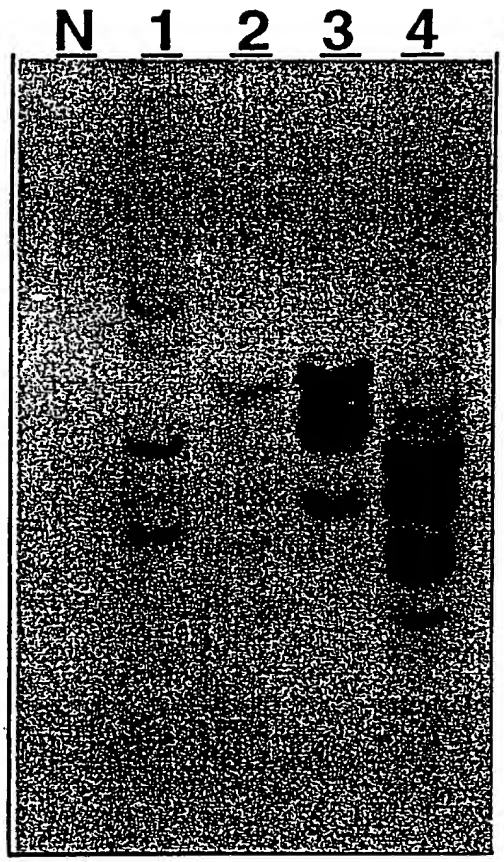


FIGURE 5

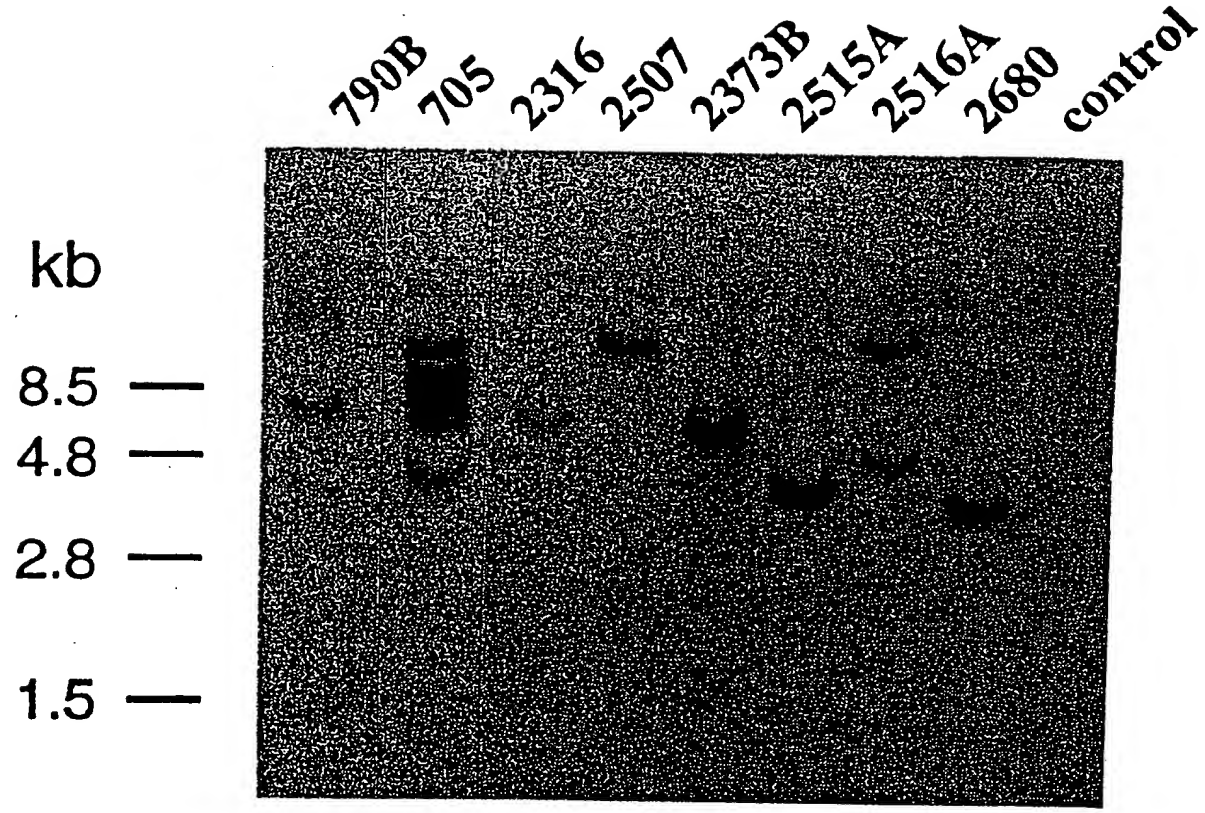
790B
705
2316
2507
2373B
2515A
2516A
2680
control
kb
8.5
4.8
2.8
1.5

FIGURE 6

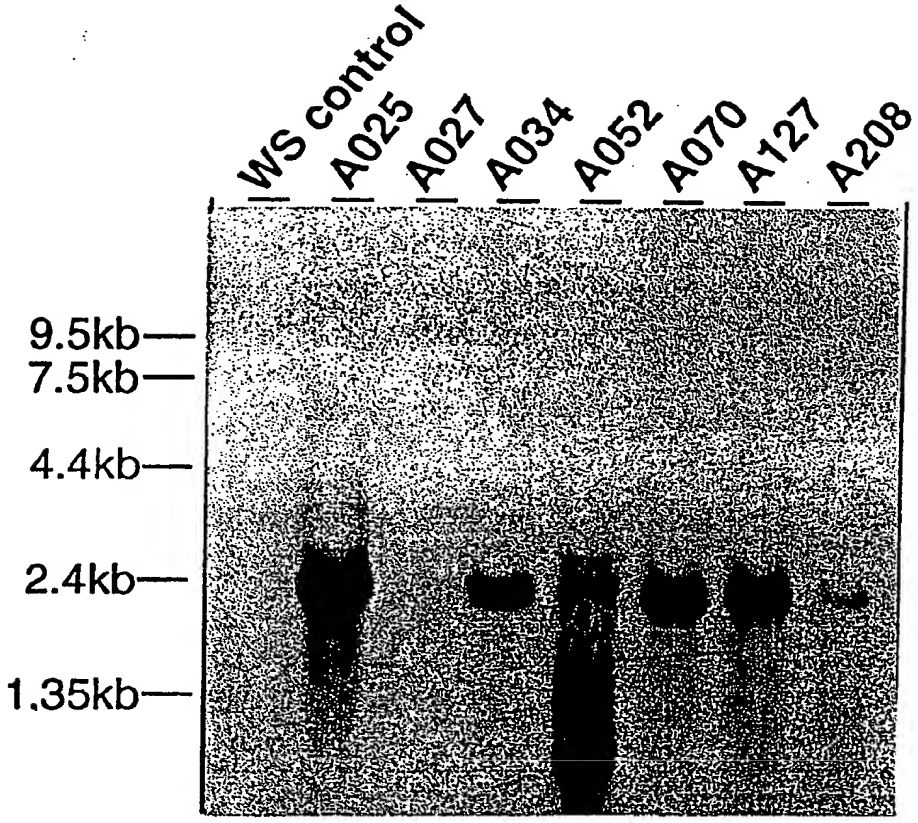
WS control
A025
A027
A034
A052
A070
A127
A208
9.5kb
7.5kb
4.4kb
2.4kb
1.35kb

FIGURE 7

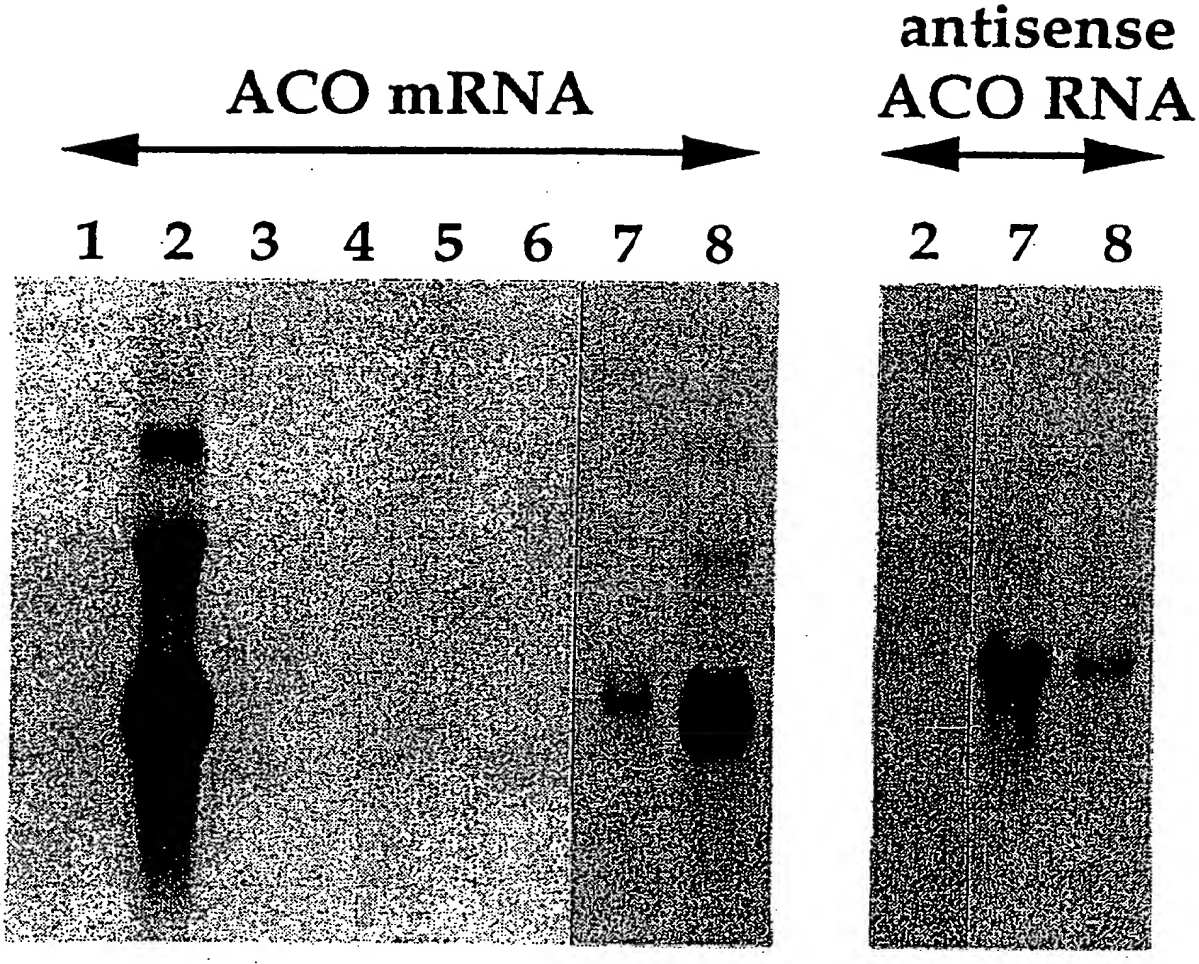


FIGURE 8

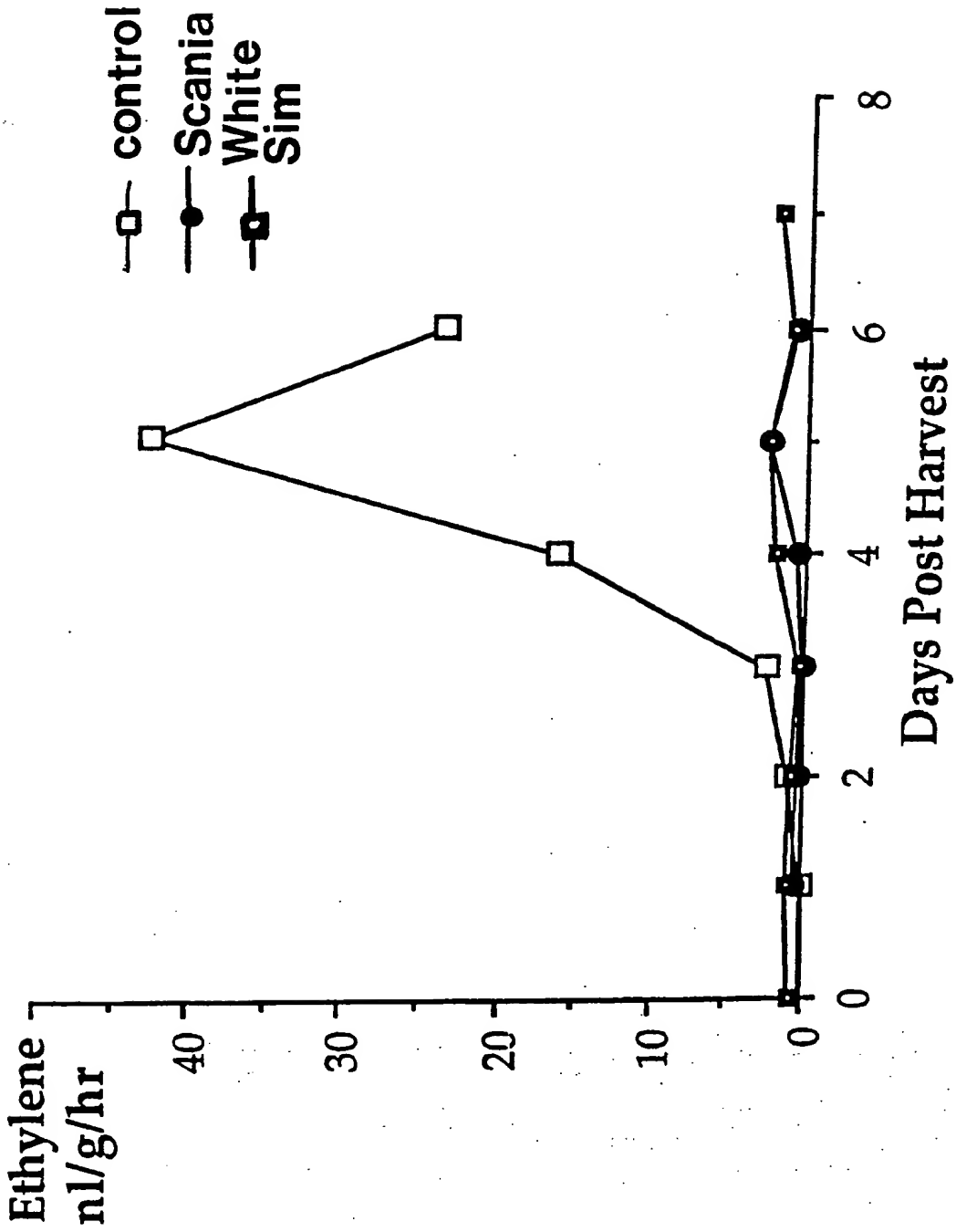
control
Scania
White
Sim
Ethylene
nl/g/hr
0
10
20
30
40
0
2
4
6
8
Days Post Harvest

FIGURE 9

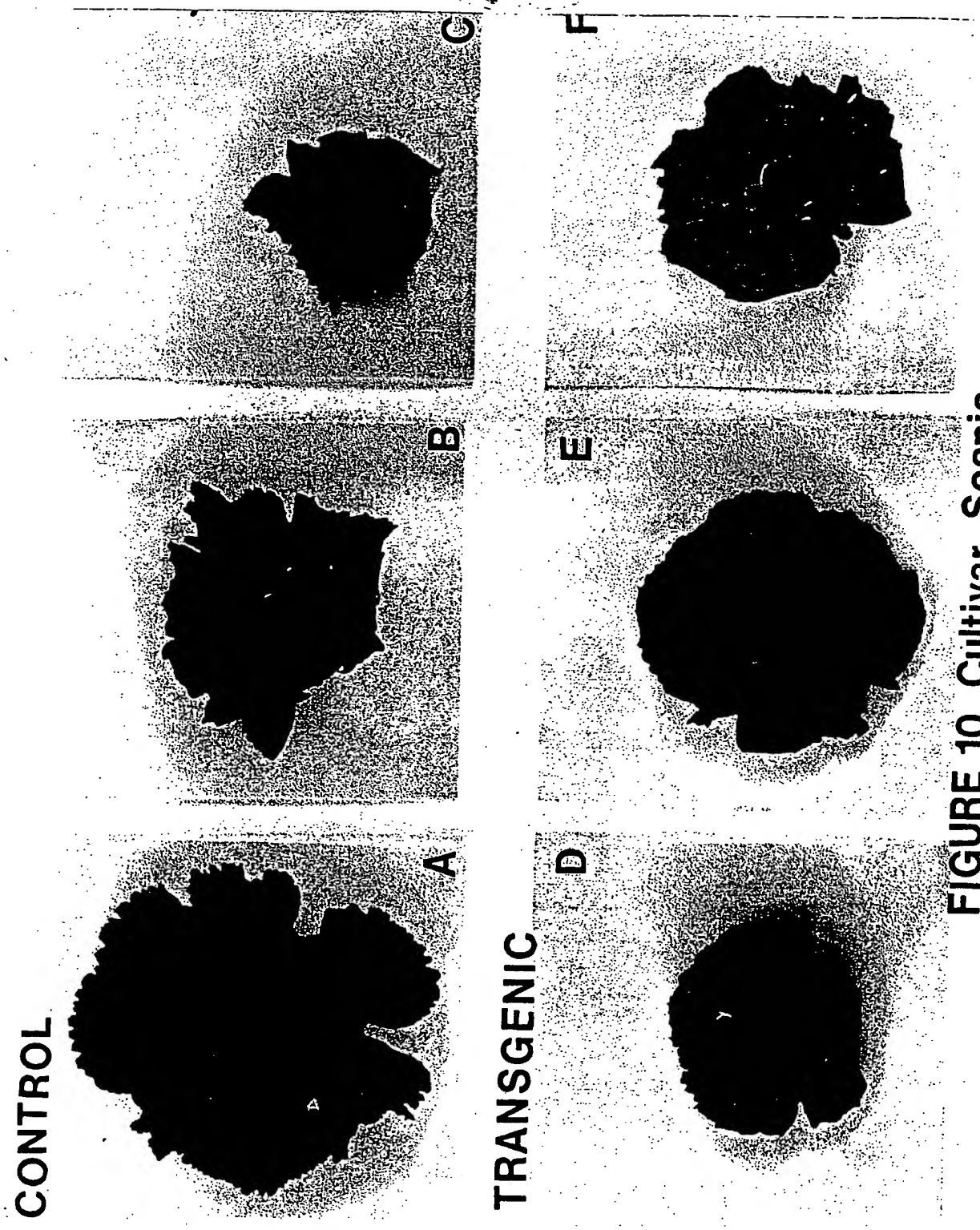


FIGURE 10 Cultivar Scania

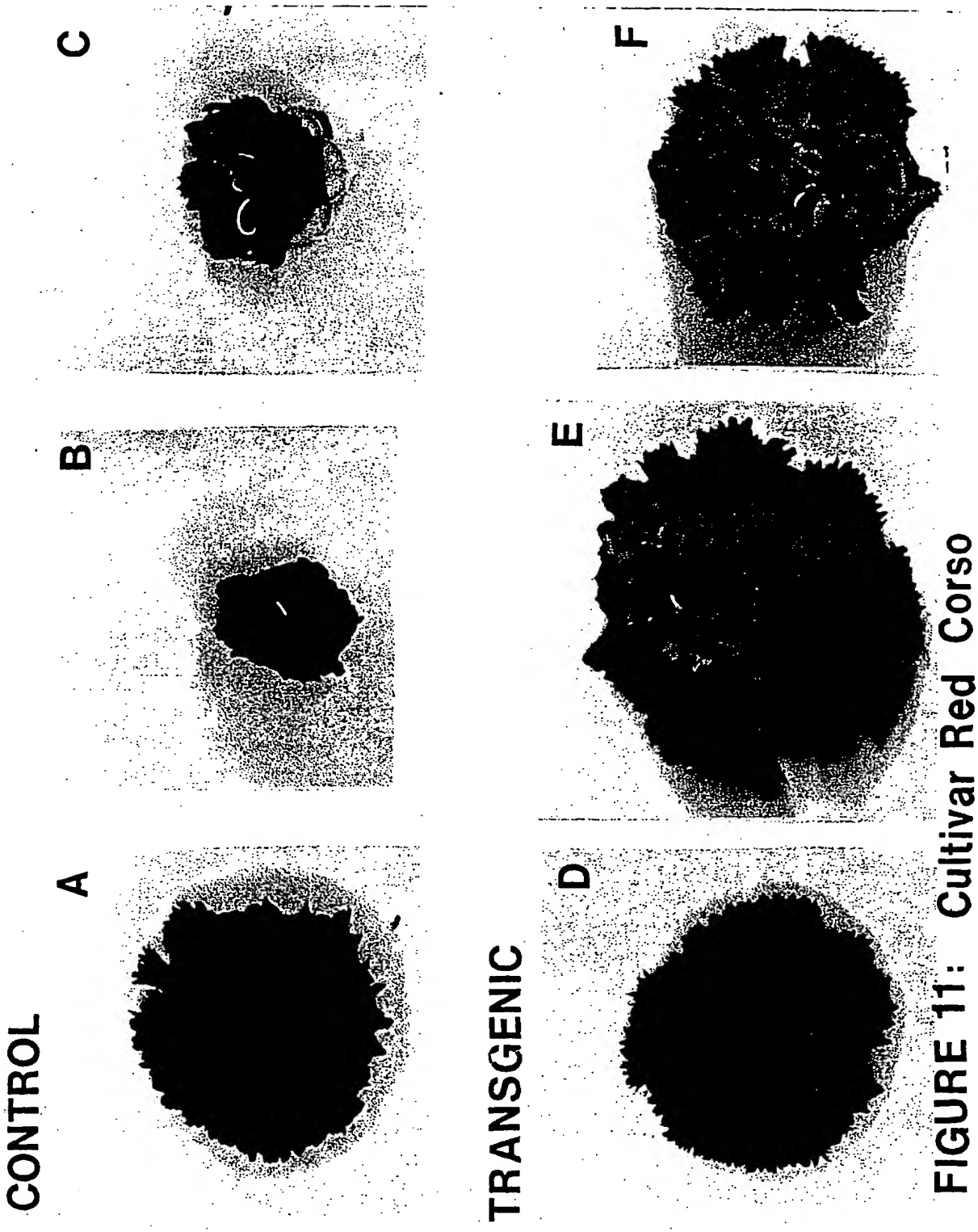


FIGURE 11: Cultivar Red Corso

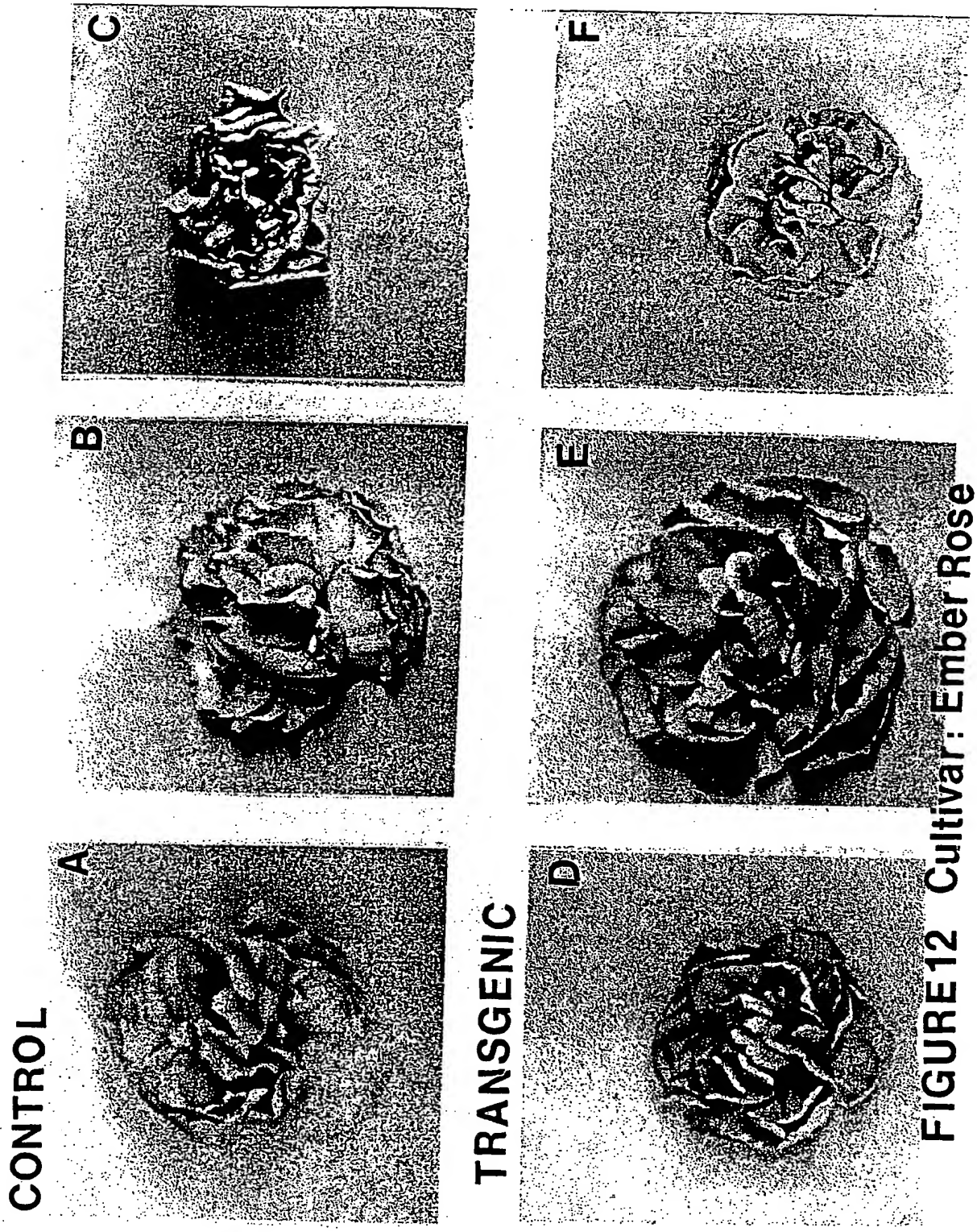


FIGURE12 Cultivar: Ember Rose

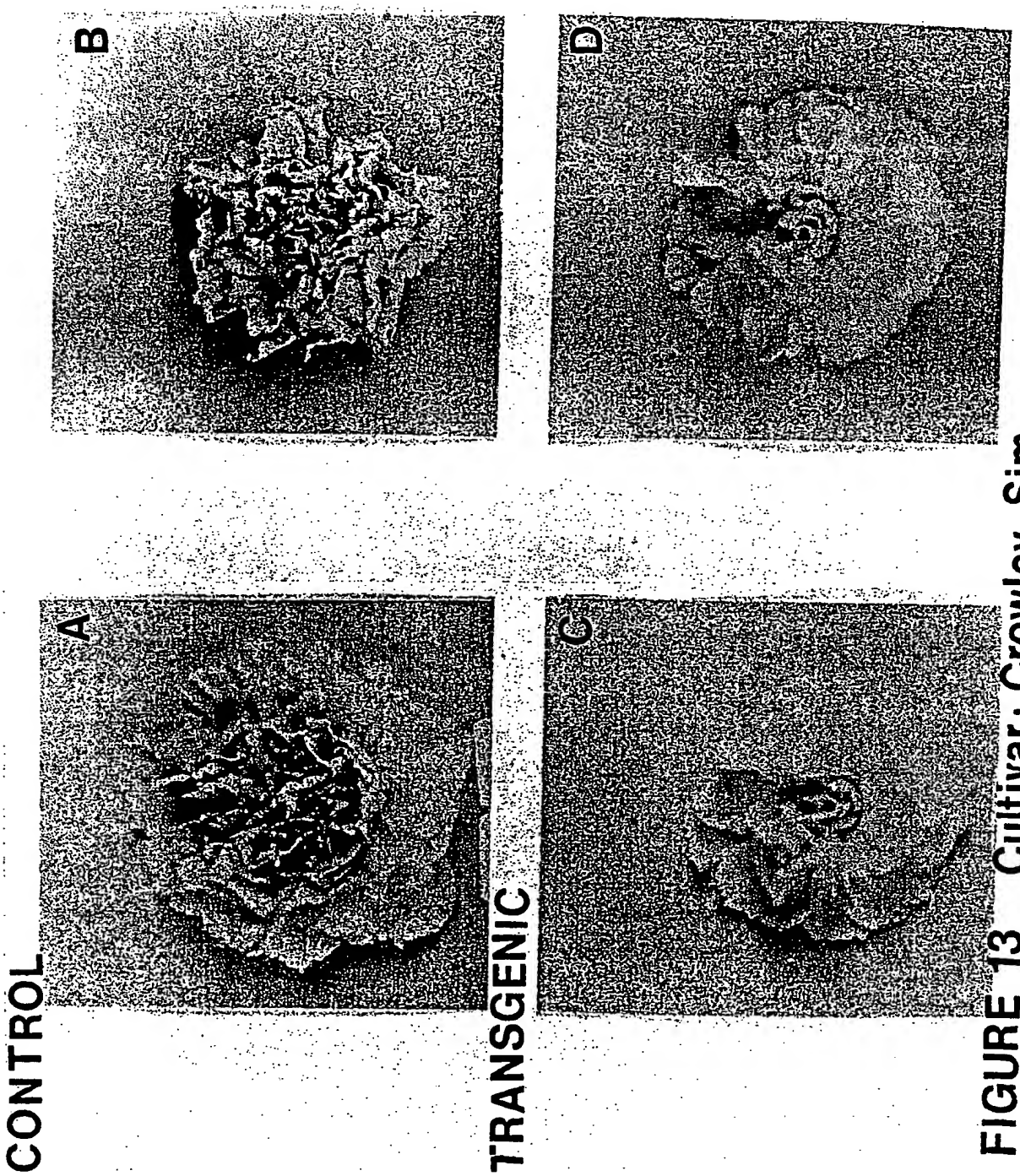


FIGURE 13 Cultivar: Crowley Sim

Day 4 post-harvest

Day 11 post-harvest

Day 20 post-harvest

FIGURE 14 Cultivar: White Sim

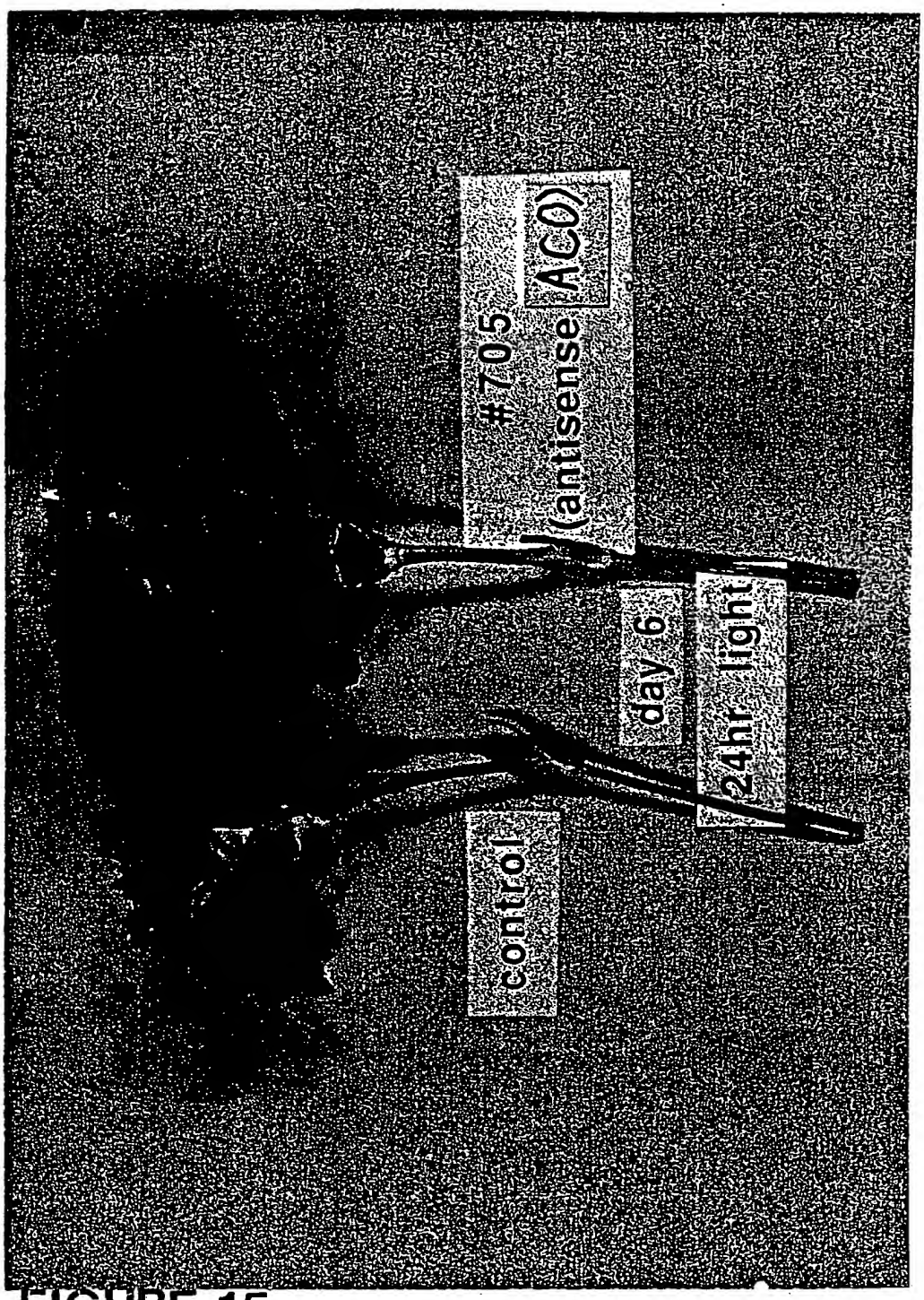


FIGURE 15

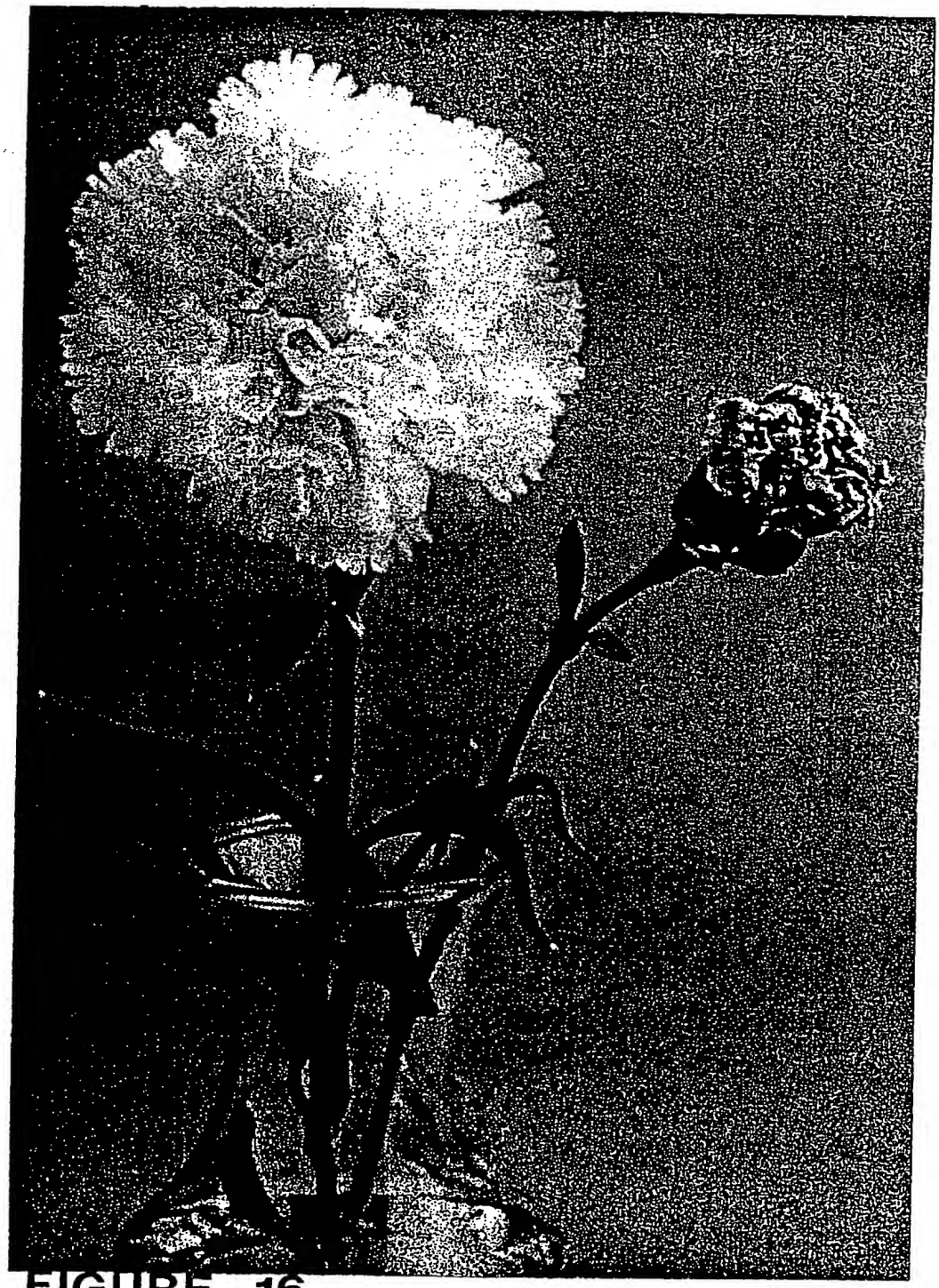

FIGURE 16

# INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 96/00286

**A. CLASSIFICATION OF SUBJECT MATTER**

Int Cl[6]: C12N 15/53, 15/60; A01H 5/02

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
Int Cl[6]: C12N 15/53, 15/60; A01H 5/02

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
BIOTECH; STN - CHEMICAL ABSTRACTS SEQUENCE SEARCH

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DERWENT - FILES WPAT, JPAT, BIOT:- SYNTHASE, AMINOCYCLOPROPANE CARBOXL: SYNTHASE, ADENOSYL METHIONINE LYASE, ETHYLENE FORMING ENZYME, ACC OXIDASE
CHEMICAL ABSTRACTS - AS ABOVE PLUS TRANSGENIC PLANT and 1991-1996/PY

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | HORTSCIENCE, Volume 29, No. 5, May 1994, K.W. Savin et al, "Delayed Petal Senescenece in Transgenic Carnation Using Antisense ACC-oxidase" page 574 abstract | 1-5, 7, 15, 23-27, 35 |
| X<br>Y | DEVELOPMENTAL GENETICS, Volume 14, No. 4, 1993, Theologis, A. et al. "Use of Tomato Mutant Constructed With Reverse Genetics to Study Fruit Ripening, a Complex Developmental Process" pages 282-295 whole document | 1-6<br>7-10, 12, 13, 15-17, 19, 20, 23-30, 32, 33, 35, 36 |

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

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Date of the actual completion of the international search
12 July 1996

Date of mailing of the international search report
29 JUL 1996

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## INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 96/00286

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| | PLANT MDL. BIOL. Volume 18, No. 2. 1992, Park, K.Y. et al. "Molecular Cloning of an 1-aminocyclopropane-1-carboxylate synthase from senescing carnation flower petals" pages 377-386 | |
| X | whole document | 1, 2, 7, 15-17, 19, 20, 23 |
| Y | | 3-6, 8-10, 12, 13, 24-30, 32, 33, 35, 36 |
| | PLANT PHYSIOL. Volume 96, No. 3. 1991, Wang, H and Woodson, W.R. "A flower senescenece-related mRNA from carnation shows sequence similarity with fruit ripening-related mRNA involved in ethylene biosynthesis" pages 1000-1001 | |
| Y | whole document | 1-8, 11, 14, 15, 18, 21, 23-28, 31, 34-36 |
| | US 5, 231, 020 (R.A. JORGENSEN and C.A. NAPOLI) 27 July 1993 | |
| Y | Whole document particularly column 7, lines 49-56; column 14 lines 38-51; claims | 1-36 |

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International Application No.
**PCT/AU 96/00286**

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

| Patent Document Cited in Search Report | | Patent Family Member | | | | | |
|---|---|---|---|---|---|---|---|
| US | 5231020 | AT | 123806 | AU | 54123/90 | DE | 69020151 |
| | | EP | 647715 | EP | 465572 | ES | 2075897 |
| | | JP | 4504800 | WO | 9012084 | | |

END OF ANNEX